VOLUME 38 SEPTEMBER 1960 NUMBER 5

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

Canadian Journal of Botany

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Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 38 SEPTEMBER 1960 NUMBER 5

A NOTE ON HYBRIDIZATION BETWEEN QUERCUS MACROCARPA MICHX. AND QUERCUS BICOLOR WILLD. IN WISCONSIN[1]

J. R. BRAY

Abstract

Collections of acorns were made in southern Wisconsin from trees identified on leaf and bark characters as *Quercus macrocarpa* Michx., *Q. bicolor* Willd., and their hybrid ×*Q. schuettei* Trel. Measurements were made of peduncle length, cup fringe length, acorn width, and acorn length. Cup fringe length and acorn length and width decreased and peduncle length increased from *Q. macrocarpa* through the hybrid to *Q. bicolor*. The hybrid was clearly separated from *Q. macrocarpa* on peduncle length. A possible introgression between ×*Q. schuettei* and *Q. bicolor* was noted.

Introduction

Many instances of apparent hybrids between *Quercus macrocarpa* Michx. and *Q. bicolor* Willd. were observed during vegetation surveys made in southern Wisconsin in the summers of 1951 to 1954. It was often difficult to separate the hybrid, ×*Q. schuettei* Trel., from its parents and a collection of acorns with attached peduncles was made to aid in identification. The present paper is an analysis of these collections.

Methods

Acorns were collected from 27 trees which occurred in 10 stands in southern Wisconsin. All collections were made from 5 to 20 August, 1954, with the exception of stand 6, which was sampled in late September 1952. All of the sampled trees occurred on the floodplain or first terrace of river valleys or in low moist meadows except stands 2, 3, 6, and 7, which were on well-drained, upland sites.

Each tree from which a collection was made was identified as either the hybrid or one of its parents on the basis of leaf and bark characters. The hybrid had leaves which widened more nearly toward the middle instead of the strong apical widening of *Q. macrocarpa*. There were deeper leaf sinuses in the hybrid than are usual for *Q. bicolor*, but a partial or complete absence of the deep central sinus of *Q. macrocarpa*. Leaves were found in which each of the lateral halves closely resembled each of the parents. Most of the hybrids observed in the field showed great variability in leaf shape and this was the

[1]Manuscript received May 6, 1960.
Contribution from the Department of Botany, University of Toronto, Toronto 5, Canada.

[The previous number of Can. J. Botany (**38**, 459–699) was issued July 22, 1960.]

TABLE I

Measurements on acorns of *Quercus macrocarpa*, *Q. bicolor*, and their hybrid

Stand No.	County	Species	Number of acorns	Mean length peduncle, mm	Mean length cup fringe, mm	Mean width acorn, mm	Mean length acorn, mm
1	Fond du Lac	*Q.m.*	9	1	2	12	11
2	Dane	*Q.m.*	11	2	2	13	16
3	Dane	*Q.m.*	7	2	4	15	13
2	Dane	*Q.m.*	7	2	3	14	17
4	Jefferson	*Q.m.*	10	2	4	15	15
5	Jefferson	*Q.m.*	2	2	5	20	19
6	Dane	*Q.m.*	5	3	4	18	20
4	Jefferson	*Q.m.*	12	3	2	11	15
7	Dane	*Q.m.*	9	4	6	18	19
6	Dane	×*Q.*	3	13	2	18	20
4	Jefferson	×*Q.*	5	13	1	13	11
1	Fond du Lac	×*Q.*	3	13	3	13	15
4	Jefferson	×*Q.*	12	14	3	16	18
5	Jefferson	×*Q.*	6	14	0	18	19
5	Jefferson	×*Q.*	12	20	0	11	10
8	Iowa	*Q.b.*?	14	21	1	13	13
5	Jefferson	*Q.b.*?	6	21	0	12	15
8	Iowa	*Q.b.*	6	21	0	12	9
9	Richland	*Q.b.*	14	26	0	9	9
5	Jefferson	*Q.b.*	6	28	1	12	13
5	Jefferson	*Q.b.*	9	29	0	12	16
8	Iowa	*Q.b.*	9	30	0	12	11
10	Jefferson	*Q.b.*	11	31	0	10	9
9	Iowa	*Q.b.*	9	38	0	9	11
5	Jefferson	*Q.b.*	10	40	0	9	11
9	Richland	*Q.b.*	9	43	1	14	12
8	Iowa	*Q.b.*	7	53	0	12	11
	Mean value	*Q.m.*	—	2	4	15	15
	Mean value	×*Q.*	—	16	1	14	15
	Mean value	*Q.b.*	—	34	0	11	11

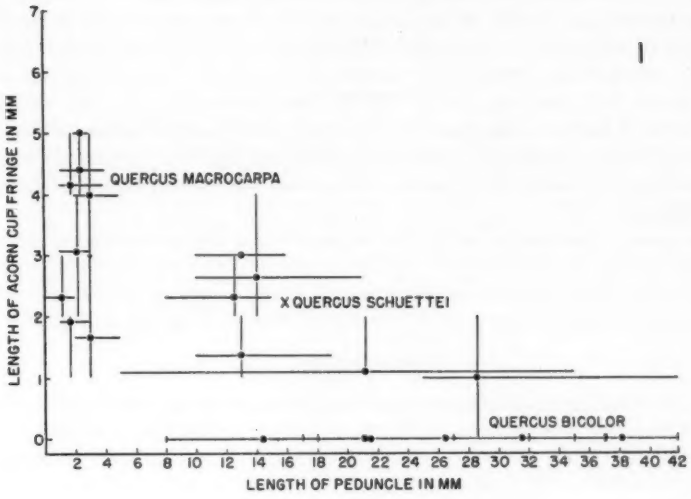
best clue to their possible hybrid nature. Bark characters were more difficult to delineate. The bark of the hybrid tended to resemble the lighter colored, more loosely fitting bark of *Q. bicolor*.

Length of peduncle, length of the cup fringe, and acorn width and length were recorded from the collections. Results, shown in Table I, are arranged in order of increasing peduncle length.

Discussion

The intermediate nature of the hybrids is clearly evident in Table I in the peduncle and cup fringe measurements. Acorn width and length decrease from *Q. macrocarpa* to *Q. bicolor*, but there is no consistent change in the ratio of width to length.

Evidence of introgression was noted from a comparison of peduncle length and cup fringe length. The pictograph technique of Anderson (1949) was used in Fig. 1 to show the nature of the variability of these characters for 20 trees selected from Table I. The lines in Fig. 1 which radiate perpendicularly from the mean value give the maximum variation for each of the two measured



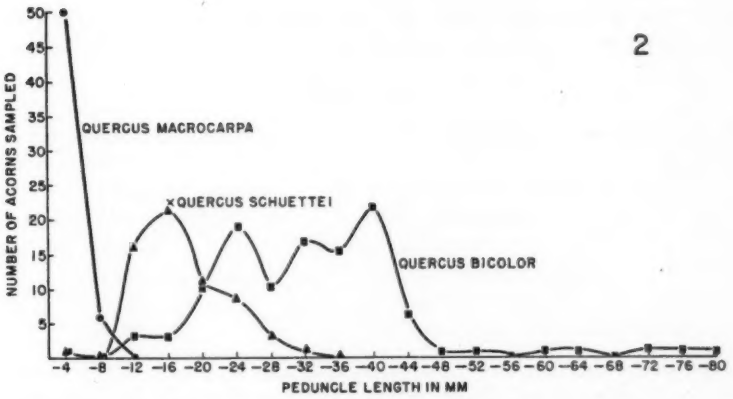


FIG. 1. Pictograph of peduncle length and acorn cup fringe length for *Quercus macrocarpa*, *Q. bicolor*, and ×*Q. schuettei*. The dots are mean averages and the lines are maximum variation around the mean. The ×*Q. schuettei* values occur from 13 to 21 mm on the *x* axis. The *Q. bicolor* values are above 21 mm on the *x* axis. The hybrid and two possibly introgressed individuals show variation in two directions, the parents only in one.

FIG. 2. Graph of frequency distribution of peduncle lengths of all sampled acorns.

values. The pictograph reveals a lack of variability in peduncle length for *Q. macrocarpa* and a lack of variability in cup fringe length for *Q. bicolor*. The hybrid is variable in both these characters, as could be expected. Introgression seems to be proceeding toward the *Q. bicolor* parent as there is a sharp break between the hybrid and *Q. macrocarpa* but not between the hybrid and *Q. bicolor*. Two *Quercus bicolor* toward the right in the pictograph have cup fringe lengths of 1 mm and a wide variability in peduncle length and may be the result of a backcross between the hybrid and the *Quercus bicolor* parent.

A frequency distribution of number of acorns in relation to peduncle length is shown in Fig. 2. The clear separation of the hybrid from *Q. macrocarpa* is evident. The distribution of peduncle length for *Q. bicolor* gives a multimodal pattern which again suggests backcrossing between the hybrid and *Q. bicolor*.

Reference

ANDERSON, E. 1949. Introgressive hybridization. John Wiley & Sons, Inc., New York.

STUDIES IN ASCOCHYTA PISI LIB.[1]

D. BREWER[2]

Abstract

The existence in peas of both physical and physiological resistance to attack by *Ascochyta pisi* was indicated. Infection was possible at constant temperatures ranging from 45 to 80° F. The temperature subsequent to incubation had a marked effect on infection: the appearance of the symptoms was delayed and the number and size of lesions were increased at the lower temperatures. *A. pisi* can utilize nitrate, ammonium, amide, and amino nitrogen. Growth is dependent upon both pH and the nitrogen source. A wide variety of carbon sources can be utilized by *A. pisi* but the isolates differ with regard to their abilities to utilize the different carbon sources. The pH of the medium is of importance at the initiation of pycnidial production. Exhaustion of the carbon supply is apparently a factor in the initiation of sporulation.

Introduction

Preliminary studies have given indications regarding the nature of the resistance of certain varieties of peas to attack by *Ascochyta pisi* Lib. and also the reasons for the limitation of necrotic areas to discrete spots (2). Additional work was undertaken with the object in mind of further elucidating these points. Since the limitation of colonization of most tissue appeared to be in some way related to the sporulation of the organisms, studies were carried out on the physiology and the conditions affecting sporulation of the organism.

Materials

The pea varieties employed, together with their reaction to *Ascochyta pisi*, were as follows:

Thomas Laxton	Extremely susceptible
Wisconsin Early	Very susceptible
Alaska	Susceptible
American Wonder	Moderately susceptible
O.A.C. 181	Moderately resistant
A-100	Immune under most conditions

O.A.C. 181 is a field variety of peas, whilst A-100 is a garden pea variety developed at the Central Experiment Farm, Ottawa, for resistance to *A. pisi*.

The following isolates of *A. pisi* were used:

Isolate No. 1, from pea seed from Western Canada;
Isolate No. 4, from foliage of *Vicia villosa* Roth;
Isolate No. 22, from foliage of *Vicia angustifolia* L.;
Isolate No. 30, from pea foliage from Ontario.

[1]Manuscript received November 15, 1959.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Based on part of a thesis submitted to the School of Graduate Studies in conformity with the requirements for the degree of Doctor of Philosophy.
[2]Present address: Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia.

Methods

The method of inoculation of plants was essentially the same as before (2), except that the spores were suspended in a 0.01% solution of aerosol rather than water. Isolate No. 1 was used exclusively in all infection studies.

The basal medium employed in the physiological studies was identical with that previously reported (1). The nitrogen source was always incorporated at a rate equivalent to 0.7 g nitrogen per liter. When a solid medium was required, agar was incorporated at 17 g per liter.

For the study of growth and sporulation in solid media, a small piece of inoculum was taken from the periphery of a culture of the organism growing on pea seed extract plus 2% glucose agar. All measurements and observations were made on duplicate cultures grown in the dark at 25° C for 14 days. The aerial mycelium was estimated as abundant (A), heavy (H), medium (M), light (L), or sparse (S). The sporulation observed was graded as abundant (A), very heavy (VH), heavy (H), medium (M), light (L), or occasional (Occ.).

When cultures in liquid media were made, 50 ml of the medium were placed in a 200-ml flask, autoclaved, and inoculated with a small piece of inoculum taken from the periphery of a rapidly growing culture. The cultures were incubated without shaking, in the dark, at 25° C.

Results

The Nature of Resistance

The delay in appearance of the disease in the moderately susceptible host, American Wonder, and the resistant, O.A.C. 181, when compared with the extremely susceptible, Thomas Laxton, was as much as 3 days. Three to five leaves per plant were taken at intervals subsequent to incubation in the moist chamber and examined for evidence of penetration of the cuticle by germ tubes. A delay in penetration of the order of 1 day was observed in the case of the moderately susceptible and resistant hosts. Penetrations were readily observed on all those types of host 3 days after inoculation, though the subcuticular hyphae appeared to be more advanced in the extremely susceptible host.

The numbers of penetrations on the moderately susceptible and the resistant host were only slightly reduced from the number observed on the extremely susceptible host, 3 to 4 compared to 4 to 6. Yet the numbers of lesions observed per leaf were markedly different. On the extremely susceptible host up to 10 lesions per leaf may develop, though the usual number is about 6, on the moderately susceptible host 2 to 3 lesions per leaf, and on the resistant host up to 2, but often no lesions per leaf. Thus there is a marked contrast between the penetrations occurring and the number of lesions developing on the resistant host and, to a less extent, on the moderately susceptible host. This suggests that the successful penetrations do not develop beyond this stage and that the resistance is physiological rather than physical.

No penetrations were observed on the leaves of the practically immune variety A-100. An attempt was made to introduce the organism beyond this potential cuticular barrier. Peas were grown in test tubes on moist, sterile

vermiculite, after being surface sterilized with sodium hypochlorite and soaked in sterile water. Inoculation was accomplished by wounding the stem with an inoculating needle and introducing a small amount of inoculum taken from a sporulating culture. Although many of the plants eventually grew beyond the plug of the tube, at the time of inoculation all were contained within the plugged tube. Checks were included using Thomas Laxton, American Wonder, and O.A.C. 181. In the case of the moderately susceptible American Wonder and the extremely susceptible Thomas Laxton the organism colonized the stem in such a way as to produce a large lesion, after completely encircling the stem, while in the resistant host, O.A.C. 181, the lesions were distinctly smaller. In the case of A-100 the organism grew but not beyond the area of tissue that was killed during the inoculation procedure.

Cross sections of leaves of O.A.C. 181 infected with *A. pisi* sometimes showed marked discolorations of the tissue at the point of penetration. While this discoloration persisted in relatively mature lesions, it was particularly noticeable in the smaller lesions and suggests the possibility of an active response of the host to attack by the organism. Brown discolorations associated with the ends of germ tubes were occasionally observed in surface examination of leaves of O.A.C. 181 and American Wonder, though in such cases there was no development of subcuticular hyphae.

Effect on Infection of the Temperature Subsequent to Incubation

The plants were inoculated in the usual way with a suspension of spores in 0.01% aerosol and they remained in the incubation chamber for 24 hours, after which time they were transferred to controlled environment chambers. The chambers were maintained at a relative humidity of 70% and the duration of light was 16 hours per 24 hours. The temperatures employed in these experiments were 45, 50, 60, 70, and 80° F. In all cases non-inoculated check plants were subjected to the same conditions. The growth of the non-inoculated check plants was dependent quite markedly on the temperature to which they were subjected.

The effects of the temperatures of 70 and 80° F were very striking in as much as the typical symptoms of the disease did not develop and the attack of the fungus was such as to cause the entire leaf to wilt and die. The attacks were similar on Thomas Laxton, American Wonder, and O.A.C. 181. Examination of the leaves for evidence of the manner in which the colonization of the entire leaf had occurred gave no results, it being difficult to locate the fungus at all. However, the presence of the fungus in such leaves was established by isolation from them. The first noticeable signs of attack were observed on Thomas Laxton from 4 to 5 days after inoculation and after 6 to 7 days on American Wonder and O.A.C. 181. These temperatures had no effect on the immunity of A-100.

At 60° F the symptoms were of the typical sort and were observed on American Wonder and O.A.C. 181 nine days after inoculation. The attack on Thomas Laxton, first observed 5 to 6 days after inoculation, was very severe at this temperature and portions of the plant were killed.

The rate of development of lesions at 50° F was slower, so that the first signs of infection were 2 days later than at 60° F. The killing of the tissue in the center of the lesion was slower and the affected area appeared as a gray colored area for some time prior to the development of the typical light-brown to tan coloration. The stem lesions that developed on O.A.C. 181 were larger than at the higher temperatures. A similar situation existed with the plants of American Wonder, where the lesions completely girdled the stem. The attack on Thomas Laxton was slowed down sufficiently for the development of typical lesions before the death of the leaf and the number of leaves killed outright at this temperature was reduced. The number of lesions that developed on American Wonder and O.A.C. 181 was greater at this temperature than at 60° F. This increase in the number of lesions at the lower temperature suggests that there may be some active reaction of the host to attack by the organism and that this reaction is adversely affected by the lower temperature. The increase in size of the stem lesions could be explained by the adverse effect on the inherent active resistance of the host to colonization by the organism.

The delay in the development of the disease was even greater at the temperature of 45° F, the difference being as great as 5 days as compared with the rate at 60° F. There was no further significant increase in the number of lesions, but the size was often much greater. On American Wonder the size of some of the lesions was such that they coalesced, a situation that seldom occurred at the higher temperature on this host. A similar, though not so pronounced, effect was observed on O.A.C. 181. In the lesions produced at this temperature there was a suppression and, at times, a complete lack of the dark-brown margin of the typical lesion. In lesions almost devoid of this colored margin, some discoloration was frequently present adjacent to larger veins. Sporulation, though not completely suppressed at this temperature, was very much delayed.

The variety A-100 was included in all the experiments and its immunity proved stable at all temperatures.

Nitrogen Utilization by the Fungus

Some exploratory studies were made respecting the nitrogen nutrition of *Ascochyta pisi* employing the following sources of nitrogen in a solid medium: potassium nitrate, sodium nitrate, ammonium tartrate (neutral), asparagine, peptone, casein, ammonium chloride, and ammonium nitrate. The results are listed in Table I.

All isolates grew well on the media containing either sodium or potassium nitrate as the source of nitrogen, though the amount of aerial mycelium produced was not as great as that produced on the medium containing ammonium tartrate. The isolates maintain their differences in rate of radial growth and also the differences in amounts of aerial mycelium on all three media. Growth was good for all isolates when either casein or peptone was the sole source of nitrogen and again the differences between isolates were essentially maintained. The mycelial mat that was produced on the medium containing ammonium chloride, though it was heavy, was atypical in that it was very much compressed and almost black in color. The colony size was also markedly

TABLE I

Growth and sporulation of *Ascochyta pisi* in culture in relation to sources of nitrogen

Nitrogen source	Isolate No.											
	1	4	22	30	1	4	22	30	1	4	22	30
	Diam. of colony, mm				Amount of mycelium				Sporulation			
$NaNO_3$	84	90	48	76	H	L	L	M	—	—	—	—
KNO_3	88	90	49	88	H	S	H	H	Occ.	—	—	Occ.
NH_4Cl	15	22	18	13	H*	H*	H*	H*	—	—	—	—
NH_4NO_3	11	45	30	17	H*	H	H	H*	—	—	—	—
$(NH_4)_2C_4H_4O$	70	75	45	80	A	M	H	A	A	L–M	—	A
Asparagine	75	85	50	75	A	H	H	A	H–VH	H	Occ.	A
Casein	70	90	40	45	H	H	H	H	L	L	Occ.	M
Peptone	90	90	50	60	H	S	H	H	A	L	—	A

*Atypical mycelium.

reduced. Isolates No. 1 and No. 30, the isolates from peas, behaved similarly when ammonium nitrate was substituted for ammonium chloride, but isolates No. 4 and No. 22, from *Vicia villosa* and *V. angustifolia*, produced some typical white aerial mycelium, though the diameters of the colonies were much reduced from those on the medium containing ammonium tartrate.

Measurement of the pH shift induced by growth of the organism in a liquid medium containing ammonium chloride showed that all isolates rapidly shifted the pH to a very low value at which no further growth could occur. This low value is about 2.9 and is reached in 7 or 8 days after inoculation of the medium. The ability of isolates No. 4 and No. 22 to produce typical white mycelium when ammonium nitrate is incorporated into the medium suggests that they are capable of utilizing the nitrate together with the ammonium nitrogen. Growth of isolate No. 4 in a liquid medium containing ammonium nitrate rapidly induced a change in the pH of the medium to a low value of about 3, yet slow growth continued for a short time. The shift induced by isolate No. 22 in this medium occurs a little more slowly than that in a medium containing ammonium chloride and after 14 days it still did not reach the limiting low pH. It is suggested that these isolates utilize both the ammonium and nitrate nitrogen at the low pH.

The growth on the media containing either potassium or sodium nitrate, though quite good, was not as great as occurred on the medium containing ammonium tartrate. The radial growth was less on the ammonium tartrate medium, but the amount of aerial mycelium was much greater than on the other two media. An examination of the pH shift in a liquid medium containing either potassium or sodium nitrate showed that the pH was rapidly increased and by the end of 2 weeks was on the alkaline side of neutrality, having started at a pH of 4.2. The movement of the pH to alkalinity in media containing nitrate salts of strong bases has been shown for many organisms that rapidly utilize nitrogen. The pH shift induced by growth in the medium containing ammonium tartrate is a lowering of the initial pH, though not as rapid as with

an ammonium salt of a mineral acid. These results suggest that the pH shift has a marked effect on growth. In solid media pH gradients readily occur and the pH in the region of the older portions of the culture may be quite different from that in the younger sections. Thus locally the medium may rapidly reach a pH unfavorable for abundant growth, yet the colony would still be able to grow in a radial direction. Such may be the case when either potassium or sodium nitrate is used as the source of nitrogen, the higher pH being unfavorable for growth. If the initial pH of either of these two media was reduced by the addition of normal hydrochloric acid, the amount of growth was increased, suggesting that pH has a marked influence on the growth of this organism on a medium containing nitrate nitrogen and that the most favorable range is below neutrality. There is also a possibility, however, that ammonium nitrogen is more readily utilizable than nitrate nitrogen, as was indicated by the type of growth observed when ammonium nitrate was used as the nitrogen source. A liquid medium was prepared incorporating both ammonium chloride and potassium nitrate, each at an amount equivalent to 0.35 g of nitrogen per liter. This liquid medium was inoculated with all four isolates and the pH shifts measured. All four isolates shifted the pH rapidly to a more acid value indicating that ammonium chloride was being utilized very rapidly. The rates of change induced by isolates No. 1, No. 4, and No. 30 were just as rapid as when ammonium chloride was employed alone, a limiting pH being reached shortly after 7 days. In the medium containing the mixture, the pH shift induced by the growth of isolate No. 22 is slower than the other three, as would be expected from its normally slower growth. The pH, however, never reached a limiting value, so growth continued slowly over a prolonged period, with the eventual production of much greater growth by this isolate. This suggests that at the low pH the organism is utilizing both ammonium and nitrate nitrogen in such proportions as to induce no shift in pH. For a short time slow growth at the low pH is shown by isolate No. 4, but it was not so great as isolate No. 22.

From these experiments it appears that growth of the organism is dependent upon both pH and the efficiency of the nitrogen source.

Carbon Utilization by the Fungus

In the preparation of the media for this series of experiments, the sources of carbon were autoclaved separately and mixed with the other components just prior to pouring the plates. The media resulting did not solidify to the same extent as when all components were autoclaved together. The reason for this is not known. The basic medium used was the same as that used in the nitrogen nutrition studies, the nitrogen source being constant, potassium nitrate, and the carbon source being variable. The carbon source was used at the 2% level and the amount of potassium dihydrogen phosphate was increased to 3 g per liter in an attempt to buffer the medium against a too rapid change to an alkaline reaction. Potassium nitrate was used as the source of nitrogen because of the possibility of the carbon in asparagine and ammonium tartrate being utilized by the organism.

TABLE II

Growth of *Ascochyta pisi* on media containing different sources of carbon

Carbon source	Isolate No.											
	1	4	22	30	1	4	22	30	1	4	22	30
	Diam. of colony, mm				Amount of mycelium (aerial)				Sporulation			
D-Glucose	82	89	60	88	H	L	M	H	—	—	—	—
D-Fructose	86	86	61	90	H	S	M	H	—	—	—	—
D-Galactose	86	88	65	89	H–A	S	H	H	—	—	—	—
D-Mannose	86	89	59	86	H	S	M	H	—	—	—	—
D-Xylose	82	84	54	76	H	M	M	M–H	—	—	—	—
L-Arabinose	86	90	54	83	M	—	M	M	—	—	—	—
Rhamnose	75	81	35	73	M	L–M	M–H	M–H	M	—	—	L
Sucrose	86	88	58	86	A	S	M–H	H	—	—	—	Occ.
Maltose	86	87	63	85	H	S–L	M–H	H	—	—	—	—
Lactose	83	87	58	79	H	L–M	M	M	—	—	—	—
Melibiose	81	90	53	82	H	—	S–L	M	—	—	—	M
Cellobiose	82	84	61	83	H	M–H	M	M	—	—	—	L–M (in center)
Trehalose	82	90	58	82	M–H	—	M–H	M–H	—	—	—	—
Raffinose	90	90	57	81	H	—	M	H	L–M (in center)	—	—	M–H (in center)
Starch	87	88	68	83	M	—	H	M	Occ.	—	—	H
Dextrin	89	90	68	90	M	—	L–M	L	M	—	—	L–M
Inulin	82	90	60	77	L–M	—	M–H	S	H–A	—	—	M–H
Sorbitol	77	84	36	79	M–H	—	M	L–M	Occ.	—	—	—
Mannitol	88	85	51	83	H	S	A	M	M	—	—	M–H
Dulcitol	—	79	43	—	—	—	—	—	—	—	—	—
Adonitol	84	88	53	78	H	L	L	L	L	—	—	—
Glycerol	85	90	50	90	S	S	L	S	L	—	—	Occ.

It was found that the isolates of *A. pisi* employed in these studies could utilize a wide variety of carbon sources and that differences could be seen amongst the isolates with regard to their abilities to utilize these different carbon sources (Table II). The differences in rates of radial growth of different isolates, previously observed on pea extract plus dextrose agar, were maintained through the series. The lower production of aerial mycelium characterizing isolate No. 4, which was first observed on pea extract agar, was also observed on the media employed here.

All isolates can utilize the monosaccharides to produce good growth, though the pentoses, xylose and arabinose, and the methyl pentose, rhamnose, do not support such good growth of isolates No. 1, No. 22, and No. 30 as do the hexoses. However, in the case of isolate No. 4, the isolate from *V. villosa*, growth is better on the medium containing xylose than on the other media. Good growth was shown by all isolates employing disaccharides as the source of carbon, though differences can be observed between the isolates when considering the amounts of growth on the different disaccharides. The greatest amount of growth of isolate No. 1 on a medium containing a disaccharide was on that containing sucrose. The best growth of isolate No. 4 was on the medium incorporating cellobiose. All isolates were able to utilize the trisaccharide raffinose as a source of carbon. Isolates No. 1, No. 22, and No. 30 were able to produce

as much growth on a medium containing this source of carbon as on those containing glucose. Isolate No. 4, however, could not utilize this source of carbon as well as the monosaccharides.

Starch, dextrin, and inulin could be used as sources of carbon for all isolates, but isolate No. 22, the isolate from *V. angustifolia*, differed from the other three isolates in that it produced more abundant growth on starch than any other source of carbon employed and that the amount of growth on the medium containing inulin was greater than on most of the other media containing either a monosaccharide or a disaccharide.

Mannitol was the best alcohol source of carbon for isolates No. 1, No. 22, and No. 30, but adonitol was the best for isolate No. 4. The vetch isolates, No. 4 and No. 22, were able to utilize dulcitol to a limited extent, whereas the pea isolates produced no growth at all when this was the sole source of carbon.

The lack of sporulation on the media which supported good growth, such as those containing sucrose, dextrose, and maltose, and the occurrences of sporulation on those media supporting less mycelial development, such as starch, dextrin, inulin, and mannitol, suggest that initiation of sporulation may be linked with the lack of readily available carbon. It is possible that the abundant growth of the organisms on solid media may lead to local exhaustion of carbon in the colony area and that when this reaches a limiting value sporulation would occur. If this is the case, then sporulation can be expected to occur after rapid growth whenever the carbon reaches a lower limiting value, providing other factors are not inhibitory.

Conditions Affecting Sporulation

Preliminary studies were made by growing the four isolates on pea plus 2% dextrose agar under continuous darkness and under alternating day and night conditions in the laboratory. It was found that the amount and character of sporulation was the same under both sets of conditions.

In view of the demonstrated ability of isolates No. 1, No. 4, and No. 30 to sporulate on the medium containing ammonium tartrate as the source of nitrogen (Table I) and the fact that the pH of the liquid medium containing this source of nitrogen drifted slowly to a low value with growth, it was thought that a relatively low pH may be conducive to sporulation in the organism. To check this, agar media were prepared with various sources of nitrogen and increasing amounts of potassium dihydrogen phosphate. To determine the effects of a higher initial pH on sporulation and growth of the organism, potassium monohydrogen phosphate was incorporated in other media in place of potassium dihydrogen phosphate.

In Table III, which is a record of observations made in these studies, +1, 2, and 3 KH_2PO_4 signify that the medium contains 1, 2, and 3 g of potassium dihydrogen phosphate per liter. Likewise + 1 K_2HPO_4 signifies 1.3 g potassium monohydrogen phosphate per liter, while + 2 and 3 K_2HPO_4 signify twice and three times this amount.

On all media containing ammonium chloride or ammonium dihydrogen phosphate, the isolates produced the atypical aerial mycelium and the smaller

TABLE III

Amount of sporulation after 14 days on media containing different sources of nitrogen and different amounts of KH_2PO_4 and K_2HPO_4

Source of nitrogen and amount of phosphate	Isolate No.		
	1	4	30
KNO_3 + 1 KH_2PO_4	Occ.	—	Occ.
KNO_3 + 2 KH_2PO_4	—	H	L
KNO_3 + 3 KH_2PO_4	VH	VH	VH
KNO_3 + 1 K_2HPO_4	—	—	—
KNO_3 + 2 K_2HPO_4	—	Occ.	—
KNO_3 + 3 K_2HPO_4	—	Occ.	—
$NaNO_3$ + 1 KH_2PO_4	—	—	—
$NaNO_3$ + 2 KH_2PO_4	Occ.	Occ.	Occ.
$NaNO_3$ + 3 KH_2PO_4	VH	VH	VH
$NaNO_3$ + 1 K_2HPO_4	—	—	—
$NaNO_3$ + 2 K_2HPO_4	—	—	—
$NaNO_3$ + 3 K_2HPO_4	—	—	—
$(NH_4)_2C_4H_4O_6$ + 1 KH_2PO_4	A	Occ.	A
$(NH_4)_2C_4H_4O_6$ + 2 KH_2PO_4	A	A	A
$(NH_4)_2C_4H_4O_6$ + 3 KH_2PO_4	A	Occ.	A
$(NH_4)_2C_4H_4O_6$ + 1 K_2HPO_4	A	A	A
$(NH_4)_2C_4H_4O_6$ + 2 K_2HPO_4	A	A	A
$(NH_4)_2C_4H_4O_6$ + 3 K_2HPO_4	VH	Occ.	A

colony size, previously noted on a medium containing ammonium chloride, except for slight amounts of aerial mycelium produced by isolate No. 22 when the medium containing ammonium dihydrogen phosphate also contained 3.9 g of potassium monohydrogen phosphate per liter. Although typical and fairly heavy growth of all isolates occurred on media containing diammonium phosphate and either 2.6 or 3.9 g of potassium monohydrogen phosphate per liter, no sporulation was observed to occur.

Isolate No. 22 did not produce any pycnidia on any of the media employed.

Isolates No. 1, No. 4, and No. 30 sporulate on all media employing ammonium tartrate as the source of nitrogen and any amount of potassium dihydrogen phosphate or potassium monohydrogen phosphate, though on the media containing 1 and 3 g of potassium dihydrogen phosphate and 3.9 g of potassium monohydrogen phosphate the sporulation of isolate No. 4 is sparse. Abundant sporulation of isolates No. 1 and No. 30 on media containing sodium or potassium nitrate did not occur unless the medium also incorporated 3 g of potassium dihydrogen phosphate per liter. Again isolate No. 4 differed in that, in addition to the abundant sporulation on these two media, heavy sporulation also occurred on the medium containing 2 g of potassium dihydrogen phosphate and potassium nitrate as the nitrogen source.

That the abundant sporulation on media containing either potassium or sodium nitrate and 3 g of potassium dihydrogen phosphate per liter was not due to the increase in phosphate content was shown by the non-production of pycnidia on media containing the equivalent amount of phosphate supplied as potassium monohydrogen phosphate. This, together with the fact that sporulation occurred on all media containing ammonium tartrate, suggests that pH is of importance in the initiation of sporulation and that the critical

value is below 7. To ascertain the trends in pH shifts induced by growth with different sources of nitrogen and different amounts of potassium dihydrogen phosphate and potassium monohydrogen phosphate, an experiment was undertaken in which liquid media were used instead of solid media. Isolate No. 1 was the only isolate used, on the assumption that the trends would be the same with the other isolates. The results listed in Table IV show that the pH change in the media with ammonium tartrate was small in any instance and that, in the media containing either sodium or potassium nitrate and 3 g of potassium dihydrogen phosphate per liter, the pH change lagged behind that in media containing the same source of nitrogen and differing amounts of potassium dihydrogen phosphate and potassium monohydrogen phosphate, though it eventually did reach alkalinity. This tends to substantiate the postulation that pH of the medium is of importance at the initiation of sporulation.

Thus the indications are that pH of the medium is a factor of importance at the initiation of pycnidial production.

Isolate No. 4 sporulated abundantly on the media containing ammonium tartrate and either 2 g of potassium dihydrogen phosphate or 2.6 g of potassium monohydrogen phosphate per liter, whereas it sporulated sparsely on all other media containing ammonium tartrate, except that containing 1.3 g of potassium monohydrogen phosphate. This suggests that there is a stimulatory effect of increased phosphate content of the medium, but that increase beyond a certain point is inhibitory. It appears that this inhibitory effect may be governed by the pH of the medium, since abundant sporulation occurs on media containing either potassium or sodium nitrate and 3 g of potassium dihydrogen phosphate per liter, media in which the pH is raised by growth of the organism.

TABLE IV

pH change in liquid media containing different sources of nitrogen and different amounts of potassium dihydrogen phosphate and dipotassium phosphate

Medium	Initial pH	pH after: 7 days	10 days	13 days	16 days
$KNO_3 + 1\ KH_2PO_4$	4.4	6.1	6.7	7.2	7.8
$KNO_3 + 2\ KH_2PO_4$	4.4	5.5	6.1	6.5	6.9
$KNO_3 + 3\ KH_2PO_4$	4.0	5.3	6.0	6.4	6.9
$KNO_3 + 1\ K_2HPO_4$	6.3	6.5	6.9	7.2	7.2
$KNO_3 + 2\ K_2HPO_4$	6.3	6.5	6.8	7.0	7.3
$KNO_3 + 3\ K_2HPO_4$	6.3	6.4	6.6	6.8	7.1
$NaNO_3 + 1\ KH_2PO_4$	4.4	5.9	6.6	7.0	7.2
$NaNO_3 + 2\ KH_2PO_4$	4.4	5.5	6.2	6.5	6.9
$NaNO_3 + 3\ KH_2PO_4$	4.3	5.5	6.1	6.4	6.8
$NaNO_3 + 1\ K_2HPO_4$	6.4	6.6	7.0	7.1	7.2
$NaNO_3 + 2\ K_2HPO_4$	6.3	6.5	6.7	7.0	7.1
$NaNO_3 + 3\ K_2HPO_4$	6.3	6.6	6.6	6.8	7.1
$(NH_4)_2C_4H_4O_6 + 1\ KH_2PO_4$	5.5	5.0	4.3	4.1	4.5
$(NH_4)_2C_4H_4O_6 + 2\ KH_2PO_4$	5.3	5.0	4.2	4.0	5.1
$(NH_4)_2C_4H_4O_6 + 3\ KH_2PO_4$	5.3	5.1	4.2	4.0	4.2
$(NH_4)_2C_4H_4O_6 + 1\ K_2HPO_4$	6.7	5.5	4.3	4.1	5.2
$(NH_4)_2C_4H_4O_6 + 2\ K_2HPO_4$	6.7	6.3	4.5	4.1	6.4
$(NH_4)_2C_4H_4O_6 + 3\ K_2HPO_4$	6.8	6.5	4.8	4.2	6.8
$(NH_4)_2HPO_4 + 2\ K_2HPO_4$	6.8	6.7	6.1	4.9	3.1
$(NH_4)_2HPO_4 + 3\ K_2HPO_4$	6.8	6.7	6.2	5.1	3.5

Since there was an abundant sporulation of isolate No. 4 on the medium containing ammonium tartrate and 1.3 g of potassium monohydrogen phosphate per liter and a low level of sporulation on the medium containing ammonium tartrate and an equivalent amount of phosphate as potassium dihydrogen phosphate, it is suggested that the potassium content of the medium may also have an effect on sporulation.

An interesting point arises when the sporulation which occurred on the medium containing potassium nitrate and 3 g of potassium dihydrogen phosphate is compared with that on the identical, but less solid, medium employed in the carbon utilization studies. In the less solid medium a more rapid diffusion of the dextrose might prevent the local exhaustion of carbon supply that was suggested as being of importance in initiation of sporulation. This depletion may be delayed until the culture is much older and then some other factor, such as pH or staling, may become the limiting factor. This indication that less solid media delayed or prevented sporulation was further examined. A medium was prepared with ammonium tartrate as the source of nitrogen, but the amount of agar was reduced to 5 g per liter. Though this medium was more solid than the media used in the carbon utilization experiments, it was found that sporulation was delayed in both isolates No. 1 and No. 30 and that even after 14 days, it was confined to the middle portion of the culture rather than being scattered throughout, as was the case on the same medium made up with 17 g of agar per liter.

Though local exhaustion of carbohydrate thus seems to have an effect on initiating sporulation, however, if the dextrose content of the medium is reduced initially to such an extent as to markedly reduce the mycelial growth, the sporulation is reduced, though not in proportion. It appears, therefore, that good vegetative development is prerequisite in culture before abundant sporulation will occur. When the dextrose content of the medium containing potassium nitrate and 1 g of potassium dihydrogen phosphate is decreased from 2 to 1%, the sporulation of isolates No. 1 and No. 30 is greatly increased and the amount of mycelial growth decreased. This suggests that, with a lower initial dextrose content, local exhaustion of carbohydrate to a critical concentration for sporulation occurred with less growth and that, due to the decrease in growth, the pH of the medium was not altered beyond the critical value for the initiation of pycnidial formation prior to the exhaustion of carbohydrate.

Discussion

The penetration studies have shown that, whereas the immunity of the variety A-100 is due to the physical barrier of the cuticle, the resistance of the variety O.A.C. 181 is not predominantly physical in nature. The cuticle does play some part in the delay of symptom expression on a resistant host. However, it is not altogether responsible for the delay, since, if this were the case, the maximum delay would be of the order of 1 day, rather than the observed maximum displacement of 3 days.

The physiological resistance of O.A.C. 181 could be affected by varying temperature to which the plants were subjected following incubation. At the lower temperatures the numbers of lesions developing per leaf were significantly greater than at the higher temperatures, though the numbers observed were never greater than the number of penetrations that had been observed on the leaves of this host and further lowering the temperatures only served to increase the size of the lesions. Thus it can be seen that the variety O.A.C. 181 possesses both physical and physiological resistances to attack by *Ascochyta pisi*.

Previously it was suggested that the limitation of colonization was due mainly to the developmental rhythm of the organism (2), as was suggested also for *Septoria lycopersici* (3). Recently it has been suggested that the reason for the production of the discrete lesions by *Septoria glycines* (4) was that at the onset of sporulation a phytotoxin was produced, which initiated a reaction in the host beyond the region of colonization culminating in the production of a zone that acted as a barrier to further colonization by the fungus. An alternative suggestion as to the mechanism of the limitation of the lesion produced by these pycnidiaceous fungi is that the colored plesionecrotic zone that is apparent at the edge of the lesion is not a reaction to a diffusible substance produced by the fungus but rather a reaction to the necrotic cells of the lesion. The production of this zone in advance of colonization at this time could be due to the onset of sporulation retarding the vegetative growth sufficiently for the formation of this zone prior to colonization by the fungus.

That the vegetative growth of the fungus either ceases or slows down at the onset of sporulation was indicated by the depletion of the protoplasmic content of the hyphal tips at the edges of the lesions at this time. The affected zone around the lesion could then serve as a barrier to further colonization as suggested by McNeill and Zalasky. Occasionally a much less vacuolate hyphal tip was seen at the edge of mature lesions suggesting that further outward growth might be possible were it not for the presence of this barrier. This is further supported by the rare observation of a hyphal strand that appeared to have negotiated the barrier to a very limited extent. In all interpretations of this phenomenon of the production of discrete lesions the emphasis has been upon the effect of the initiation of sporulation. Thus the increase in size of the lesions observed at lower temperatures can be readily explained by the retardation of sporulation, an effect that was seen, rather than by an effect on the defense mechanism of the host.

The results of the nutritional experiments have shown that there is a marked difference between the vetch and the pea isolates with regard to their ability to utilize various carbon sources. Both pH and carbon supply appear to be of great importance in the initiation of sporulation, though the indications from the behavior of isolate No. 4 are that, in the case of this isolate, both the potassium and phosphate content of the medium also play an important role. Thus again an isolate from vetch differs somewhat from the isolates from peas.

Acknowledgments

The author wishes to express his sincere gratitude to Professor D. L. Bailey for his guidance, encouragement, and helpful criticism throughout the course of this work.

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A DEVELOPMENTAL STUDY OF WILD RICE, ZIZANIA AQUATICA L.[1]

CYNTHIA E. WEIR AND HUGH M. DALE

Abstract

The life history of wild rice is traced from the appearance of the first submersed leaves in the spring until the seed of this annual plant is buried at the bottom of a lake in the fall. The characteristics of the three types of leaves are discussed. The change from the simple vegetative apex to the many-tipped, young inflorescence occurs early. Thus the potential yield in rice grains is determined as the tips of the upright leaves reach the surface of the water and before the stem elongates. The inflorescence develops acropetally as does each spikelet. Only at the later stages can the pistillate and staminate spikelets be distinguished. The development of the microspores and the nuclear changes in the embryo sac have been elucidated. In *Zizania* the embryo development has been found to be a variant of that found in *Poa* while the endosperm develops chiefly from the outside. A well-differentiated abscission layer develops at the base of each spikelet.

Zizania aquatica L. is an annual plant that has been harvested by North Americans for centuries. The grain collected from natural stands was formerly regarded as 'food from the gods' and was used in religious ceremonies. Today rice is collected by many Canadians and Americans of Indian descent using simple home-made machines and it provides a valuable income for these people. In Minnesota a recent harvest was valued at $400,000 (22). As well as being part of the diet of these people, this rice is considered a delicacy by gourmets and it is also an important food for ducks in the autumn. The grain was found to be second to the very soft aquatics when the contents of stomachs of 8000 ducks in 200 different localities were analyzed (19). Twenty-six species of birds are known to feed on *Zizania* (20).

Although this plant has attracted the attention of scientists since the eighteenth century, very little has been added to the traditional facts about germination, growth, and production of grain (6, 7, 9, 25). In this paper an account will be given of the normal structure, and the development from germination to flower and seed. It is anticipated that at a later date the effects of various environmental conditions modifying this normal development will be elucidated, shedding light on the ecology of *Zizania*.

Life History

Zizania aquatica is an annual aquatic whose seeds normally sink in the mud in the fall. Early stages in seedling development have been described (2, 26). Long ribbon-like leaves are the first indication of this plant. In Southern Ontario these usually appear in the middle of May. These leaves are completely submersed, and may be seen streaming out in the water with the current. Towards the end of May the floating leaves are produced. These linear leaves are never completely submersed, but have an upper surface

[1]Manuscript received April 6, 1960.
Contribution from the Department of Botany, Ontario Agricultural College, Guelph, Ontario.

which is never wet. After the formation of one or two of these floating leaves there appears a third type which is stiff, upright, and aerial. Each of these three leaf types has a characteristic structure. Plants growing in shallow water produce fewer floating and submersed leaves than the plants growing in deeper water, and seedlings growing on damp peat moss produce only aerial-type leaves.

The depth of the water in which *Zizania* grows affects not only the type of leaves produced, but also the height of the plant and its internal structure. The height of a plant growing in 2 ft of water may be 4 ft from base to the flower heads, while a plant in the same stand, but in deeper water, may grow to a much greater height. The difference in height is due to the length of the internodes, rather than to the number of internodes. Short plants growing in shallow water, or on the banks of rivers or lakes, are found to have more heavily lignified tissue in the stem.

Flowering plants may be found from the middle of July through August. Plants occurring in the most southern parts of Ontario flower first, while flowering is delayed in northern stands, which occur as far north as 53° in Ontario. The spikelets occur on a terminal panicle and are unisexual and one-flowered, with the pendulous staminate spikelets on the middle and lower branches, while the pistillate spikelets are appressed to the upper, nearly erect branches. Figure 7 shows an inflorescence in silhouette. The upper pistillate portion is green with the spikelets closely appressed to the branches, while the lower, yellow, staminate portion consists of whorls of spreading branches which can be seen arising from the main axis. Secondary branches on these bear the pendulous spikelets. Figure 1, *a*, depicts a pistillate spikelet and Fig. 1, *b*, a staminate one. Maturation proceeds from the tip downwards and each inflorescence lasts for a number of days. The staminate spikelets begin to shed their pollen just as the last pistillate spikelets have been pollinated or have reached anthesis. Self-pollination is therefore unlikely.

Sometimes on the inflorescence at the junction of pistillate and staminate spikelets, there may be hermaphrodite spikelets, with both stamens and ovary well developed. These spikelets may produce seeds and may be self-pollinated. Figure 8 is a photograph of part of a branch of an inflorescence, showing three spikelets in which the caryopsis has begun to develop. Near the base of each of the three spikelets, the remains of the stigmatic arms can be seen projecting laterally. These are indicative of functional pistillate spikelets. The lowest spikelet, which is hermaphroditic, shows four of the six fully developed stamens which have shed their pollen as the four larger projections on the right hand side. The awns extending from the lemmas of the two upper spikelets are much longer than that of the lowest spikelet. Long awns are characteristic of pistillate spikelets, while diminutive awns are a feature of staminate spikelets. The shorter awn on the lowest spikelet is also an indication of its intermediate condition.

After pollination the spikelets take from 10 days to 2 weeks to produce fully mature caryopses, which are greenish, brown, or black in color (Fig. 1, *c* and *d*). The grains fall off as soon as they mature so that from each plant only a few

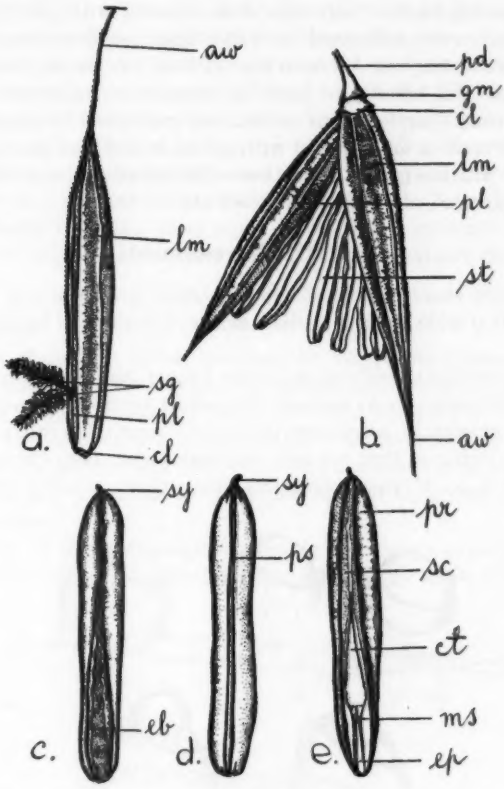

FIG. 1. *a*, Pistillate spikelet at anthesis. *b*, Mature staminate spikelet. *c*, *d*, Mature caryopsis with lemma and palea removed; *c*, embryo side; *d*, placenta side. *e*, Mature caryopsis with pericarp removed to display parts of embryo.

aw, awn; *cl*, callosity; *ct*, coleoptile; *eb*, embryo; *ep*, epiblast; *gm*, vestigial glumes; *lm*, lemma; *ms*, mesocotyl; *pl*, palea; *pd*, pedicel; *pr*, pericarp; *ps*, groove made by placental strand; *sc*, scutellum; *sg*, arms of stigma; *st*, stamen; *sy*, remnant of style.

ripe grains may be collected at one time. Therefore it is only possible to harvest part of the crop at each visit to a stand. The grain may float in water for a short time but it soon sinks.

Materials and Methods

The material studied was grown from seed or collected from populations of *Zizania aquatica* var. *angustifolia* in Southern Ontario. Shoot tips were collected from plants at intervals until fruits were produced. Some young plants were collected in the field and grown immersed in tanks in the greenhouse where they were bathed with a constant stream of water. Before the cultured plants

produced flowering heads, their tops were covered with polythene bags. At anthesis, flowers were pollinated by hand, and specimens were collected at hourly intervals on the first day, and then at intervals during the next 2 weeks.

The material was killed and fixed in formalin acetoalcohol, then passed through a tertiary butyl alcohol series and embedded in paraffin. Sections were cut at 10 or 20 μ and stained with safranin and fast green, or with iron haematoxylin. Mature grains, which were difficult to cut, were first soaked in a solution of glycerol, alcohol, and aerosol (3).

Vegetative Development

The vegetative shoot apex in *Zizania* is small, a typical one being 97 μ in length, and 103 μ wide when a median section is measured by Stant's method (31).

The leaf primordium develops as a ridge a short distance from the tip of the shoot apex. This ridge grows laterally as well as vertically, so that it forms a collar which completely surrounds the apical cone. The vertical growth of the collar is eccentric so that one side becomes higher than the other, forming a cowl. Figure 2, *a*, shows a young leaf primordium, circling the base of the

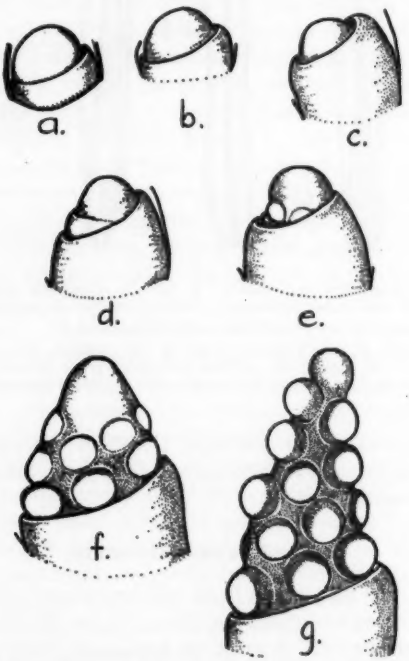

FIG. 2. Shoot apices. *a–d*, Successive stages in the formation of a leaf. *e–g*, Successive stages in the development of primordia of inflorescence on floral apex.

apex. In Fig. 2, *b*, it has grown around the apex as a partial cowl, which has further developed in 2, *c*. As growth continues, the primordium of the next leaf develops on the side of the stem opposite the one just below it, producing the normal two-ranked arrangement found in grasses (Fig. 2, *d*). Each leaf primordium grows to encircle the apex. Thus the vegetative apex becomes situated inside a number of concentric tubes, each a sheathing leaf base. The top of the cowl grows up to form the leaf blade and the sides grow past each other so that the leaf blades are imbricated.

During plastochron, the stem apex is relatively constant in shape, but elongates so that it remains above the tip of the youngest cowl as shown in Fig. 2, *c*.

Submersed Leaves

The blades of the first leaves produced by the young *Zizania* seedlings are ribbon-like. After five plastochrons the simple leaf whose initiation has been described can be subdivided into blade and sheath as by that time the ligule is initiated by the cells of the adaxial surface as described in *Zea* (28). The blade and sheath differentiate independently, a meristematic region remaining at the base of the blade above the ligule as well as at the junction of the base and the stem.

The midvein of these submersed leaves is not conspicuous but differs little from the lateral veins, all differentiating basifugally as in *Zea*. Figure 3, *a*,

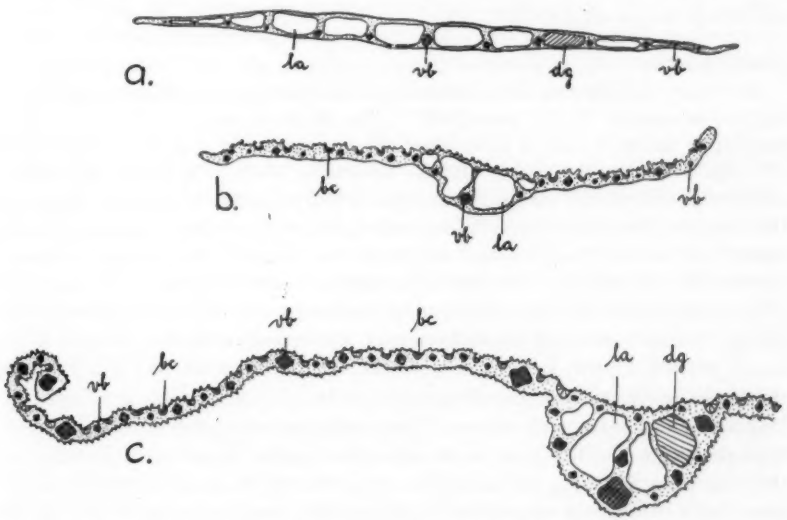

FIG. 3. Transverse sections of leaves: *a*, submersed leaf; *b*, floating type; *c*, emergent type.

bc, rows of bulliform cells; *dg*, diaphragm across lacuna; *la*, lacuna or air space; *vb*, vascular bundle.

is a diagram of a cross section of a typical submersed leaf with 12 lateral veins and a midvein. The bundles, which are devoid of sclerenchymatous caps, appear towards the lower surface of the leaf and are embedded in the strands of parenchymous tissue which separate the large schizogenous air spaces of the mesophyll. The xylem of the veins, which always occurs towards the upper surface, contains very few lignified vessels. Five out of 13 bundles in a typical leaf cross section contained two lignified xylem vessels each whilst the remainder gave no sign of lignin when tested with phloroglucinol and hydrochloric acid.

The mesophyll surrounds the large septate lacunae and forms a double layer of cells inside the upper and lower epidermis. The longitudinal strands of parenchyma cells surround the vascular bundles and are two or four cells in thickness (with a vein embedded in each) so that adjacent lacunae are well separated. Each lacuna is also chambered by a number of transverse diaphragms consisting of a double row of well-rounded parenchyma cells with prominent chinks (*dg*, Fig. 3, *a*). The chambers are approximately three or four times as long as wide.

Neither the upper nor the lower epidermis of the submersed leaves contains chloroplasts or stomata. The cells of the epidermis occur in long files. Some consist only of cells which are thin and narrow while other files have the occasional very short cell. The walls of all cells are smooth and lack the sinuosity of many epidermal cell walls. Above and below the veins, the epidermal cells are short with uniquely thickened walls. These have been reported also in the epidermis of rice, *Oryza sativa* (16).

Floating Leaves

Normally only two or three submersed leaves are produced and then floating leaves appear. In the mesophyll of the blades of each of these is a well-developed midvein with a prominent thickening of the leaf, as well as 10 or 12 large lateral veins and a number of smaller ones (Fig. 3, *b*). On either side of the midvein are air spaces, the two largest adjacent to the vein. Between the lacunae, strands of parenchyma cells surround the vascular tissue. In the central strand are two bundles, the larger one towards the abaxial or lower surface. Each bundle is enveloped in a sheath of sclerenchyma.

The lower epidermis is in every way similar to that of the submersed leaf blades, without chlorophyll, stomates, or epidermal hairs but consisting of small, smooth-walled, long cells, with the occasional short cell. The cells of the upper epidermis occur in longitudinal files, many of long cells, some of long and short cells, and others of long cells and stomata. Most cells have typical wavy walls. There are numerous cellular hairs projecting from many of the long cells. All cells, including the trichomes, have small blisters on their outer walls. These are over twice as tall as wide and are produced by a local thickening of the wall. The outer walls, like those of other aerial epidermal cells, are covered with cuticle. The roughness produced by the projections traps air bubbles and the cuticle prevents the surface from becoming wet, so that

water tends to ball up and roll off. The surface is not wet even when repeatedly submerged by waves. The guard cells of stomates are sausage-shaped and each has a large triangular accessory cell. The chlorophyll-bearing guard cells are depressed below the level of the epidermal cells. Bulliform cells are a feature of this upper surface of the floating leaf blades with 14 rows of bulliform areas across each leaf (*bc* in Fig. 3, *b*). In each row there are four or five files of these very large thin-walled cells.

Emergent Type of Leaves

After two or three floating leaves are produced the tips of the stiffer emergent leaves appear above water (Fig. 3, *c*). These leaves might very suitably be described in the terms used for those of *Oryza sativa* (16). The upper surface is similar to that of the floating leaf blade. Along the edge of the blade are sclerenchyma cells and a row of siliceous epidermal hairs. The epidermal cells are decorated with hairs, blisters, and cuticle as is the lower epidermis of these emergent blades. The presence of 46 long files of bulliform cells on the upper surface of a typical leaf seen in cross section is the most prominent difference between the upper and lower surfaces (*bc*, Fig. 3, *c*) and the 53 veins indicate a very different development from the submersed or floating leaf. In the region of the midvein are four very large air spaces separated into long canals by thin radiating layers of parenchyma. These run the length of the leaf near the midvein but are septate, being divided into compartments by transverse diaphragms of three or four tiers of star-shaped cells with large spaces. These are similar to those described in the roots (33). Each diaphragm supports transverse veins joining adjacent lateral veins or the midvein to a lateral. These diaphragms occur at intervals so that each compartment of the lacuna is from three to eight times as long as wide.

Leaf Bases

The imbricated leaf bases of *Zizania* appear similar in structure to the submersed leaf in transverse section. There are the same number of longitudinal schizogenous air spaces as in the submersed leaf separated by longitudinal strands of parenchyma cells two to four cells thick. There are also transverse diaphragms, dividing the lacunae into chambers. Embedded in the longitudinal strands of parenchyma near the abaxial epidermis are alternating large and small vascular bundles. Above each vascular bundle are two to three rows of sclerenchyma cells.

The cells of the mesophyll parenchyma are larger towards the abaxial surface consisting of two to three layers of large, round, chlorenchyma cells under the single layer of small-celled epidermis. Cells towards the adaxial surface are devoid of chlorophyll and the cells of the single parenchyma layer are diminutive or even absent in some areas. The edges of the leaf bases are strengthened and given rigidity by a file of small thick-walled cells. There is also a row of sharp upward-pointing trichomes along the leaf margins as in other grasses.

Floral Development

Transition from the Vegetative to the Floral Apex

The shoot apex changes from the vegetative to the floral type after the middle of June, when the first stiff upright leaves are produced. Internally, the transition is marked by vigorous meristematic action. The short time between divisions allows for only slight cell enlargement so that the cells are smaller and stain more densely. This increased meristematic activity causes the apical cone to increase in length at a much faster rate than the leaf primordium. The shape of the apex is modified from the hemispherical appearance indicated in Fig. 2, *a*, to the broad cone seen in 2, *f*. At the same time, due to periclinal division in the layer of cells beneath the surface, the cone becomes covered by a series of spirally arranged swellings. These are primordia of branches of the inflorescence (Fig. 2, *f* and *g*). Nearly all of these branch primordia split up to produce subdivisions of the branches, each with an active meristematic area at its tip. These are darkly stained in Fig. 9, which is part of one branch of many in the inflorescence. Each dark tip is the primordium of a single spikelet. The number of spikelets, and therefore the potential number of seeds, will depend on the number of dark swellings produced on the apex and the degree of branching. The terminal branch of the inflorescence bears only a single pistillate spikelet, while the staminate spikelets towards the base are borne in whorls on three or four branches as can be seen in Fig. 7. The floral primordia near the apex develop more rapidly and become ready for pollination first. Thus while the upper branches are differentiating, the basal branches are still producing lateral outgrowths, the primordia of spikelets.

Long, silky, multicellular hairs produced from the epidermal cells of the stalk-branches surround the developing inflorescence (Fig. 9).

Development of Staminate and Pistillate Spikelets

The early stages of development of both staminate and pistillate spikelets are identical. They develop acropetally. During the first stage a ridge surrounding the base of the vegetative cone is formed. This is followed by a second similar one, above the first. These both remain small at maturity. The lower one, the vestigial glumes, forms the cup at the tip of the pedicel into which the spikelet is inserted. The upper one becomes an eccentric collar at the base of the spikelet, called the callosity (10, 13). It is devoid of a vascular supply while the lower cup has 10 to 12 traces. This would suggest that the lower one represents two fused vestigial glumes. The vascular strands of these vestigial glumes are not equally well developed; however, in several strands sieve tubes are found, as well as more than five rows of scalariform vessel elements, some of which become lignified.

Figure 4, *a* shows an early stage in the development of any spikelet. At *vg* is the ridge which develops into the vestigial glumes indicated at later stages in Fig. 4, *b–e*. At *cl* the second-formed ridge, the callosity, is apparent (Fig. 4, *a–e*). In almost all spikelets an abscission layer is formed between the vestigial glumes and the callosity (*ab*, Fig. 4, *c*).

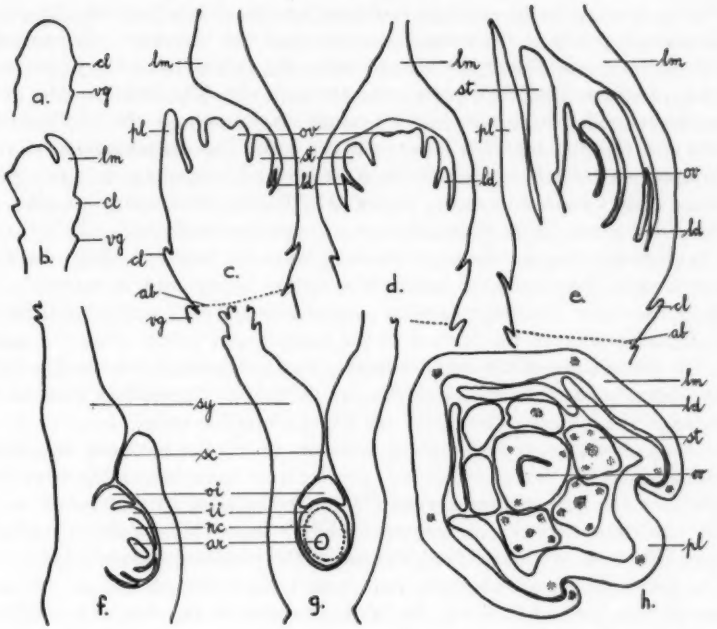

FIG. 4. Development of the spikelet shown by sectional outlines. *a*, *b*, *c*, and *e*, Successive stages in the development of a spikelet, and *d*, a section at right angles to *c*. *f* and *g*, Sections of ovary at right angles to each other. *h*, A transverse section of young spikelet *f*–*e*.

ab, abscission layer; *ar*, archesporial cell; *cl*, callosity; *ii*, inner integument; *ld*, lodicule; *lm*, lemma; *nc*, nucellus; *oi*, outer integument; *ov*, ovary; *sc*, stylar canal; *st*, stamen; *sy*, style; *vg*, vestigial glume.

The next structure to be differentiated on the apical cone of the spikelet is the lemma. This arises as a semicircular fold on the abaxial side of the main axis of the inflorescence, but adaxial to the inflorescence branch. The early development of the lemma is indicated at *lm* in Fig. 4.

The developing palea is indicated at *pl* in Fig. 4. The primordium of the palea arises later on the side of the meristematic cone opposite the lemma and just a little above its point of attachment. The palea is also crescentic in cross section, but not quite as large as the lemma. By the time the palea makes its appearance, the lemma has increased in length, and its tip tapers off to become an awn. The later developing palea becomes tightly enclosed by the margins of the surrounding lemma (Fig. 4, *h*). The lemmas of both the staminate and pistillate spikelets have a vascular supply of five strands, while the palea has three. Figure 4, *h*, shows transverse section of a spikelet in which the vascular supply of both palea and lemma are demonstrated.

The structure of both the palea and the lemma is quite simple. In the early stages of their development, they consist of an outer and an inner epidermis,

enclosing a compact layer of parenchyma four to five cells thick. As growth continues, the cells of the inner epidermis, and the layers of cells nearest it, swell up. In the pistillate lemma and palea the cell walls of the outer layers thicken, become lignified greatly, and the cells die. The swollen cells of the inner layers collapse, so that in the mature caryopsis, only the lignified outer layers and the disintegrated inner cells are seen. The disintegration of these cells is somewhat reminiscent of the formation of lysigenous air space in the roots of some Gramineae and in leaves (21, 29, 30). Although the lemma and palea of a mature pistillate spikelet are at most four cells thick, the cell walls are lignified forming a resistant protective layer for the developing embryos. Thickening in the staminate spikelets is not as heavy, and at maturity, the walls of the outer layer of cells alone are thickened. This is readily noticed in a casual examination of the texture of the lemmas and paleas of mature spikelets. On the surface of the mature lemma and palea, especially in the region of the ridges, several bristle-like hairs are produced. These hairs grow on the margins of the lemma as well, and are found along the awn.

In both pistillate and staminate spikelets of all the material of *Zizania aquatica* var. *angustifolia* examined, the lemmas have invariably been five-nerved and the paleas three-nerved. The material used by Hitchcock to describe the characteristics of the genus differs from this condition, as in his material there were three-nerved lemmas in the pistillate spikelet (13).

The two lodicules are the next structures to be differentiated at the apex. They are indicated at *ld* in Fig. 4 and can be seen on the side of the spikelet nearest the lemma. At anthesis, they are membranous with a single terminal projection which appears as a hook at the base of the stigma. Prior to anthesis, the cells of the lodicules of the pistillate spikelet swell up and assist in pushing the stylar arms between the lemma and palea. After pollination they atrophy. In the staminate spikelet the lodicules help to separate the palea and lemma, so that the mature stamens may be freed for pollen dispersal. The stamens arise after the lodicules and the six staminal primordia appear as two groups of three adjacent initials. The three that arise first are not always in the same position. In Fig. 4, *h*, the three anthers enclosed by the palea show greater differentiation. In all spikelets the stamens develop four-lobed anthers with a central vascular strand (Fig. 4, *h*). Each lobe contains a single vertical row of archesporial cells. In the pistillate spikelets disintegration takes place soon after the stages shown in Fig. 4, *e* and *h*, but in the staminate spikelets the anthers of four chambers enlarge greatly as they mature.

The primordium of the pistil arises last. First a ridge appears near the tip and almost completely surrounds the apex as is indicated in Fig. 4, *d*. This ridge elongates until it produces two conical outgrowths which later become the stylar arms seen in Fig. 4, *g*. The wall of the ovary is complete, except at the tip where it forms the stylar canal indicated at *sc* (Fig. 4, *f* and *g*).

Development of the Microsporangium

From Fig. 4, the young anthers can be visualized as arising as a fold on the apical cone after the lodicules have been initiated. In all the material examined

three adjacent staminal primordia developed earlier than the others. In no case was there evidence that alternate initials form a separate whorl. This suggests a single whorl of six stamens in *Zizania*. At the earliest stage the young anther consists of undifferentiated meristematic cells. Differentiation is first seen in cells of the second layer. They enlarge to produce the four lobes and undergo repeated division.

This original group of meristematic cells in each lobe divides periclinally to produce a layer of hypodermal cells, the primary parietal layer, which surrounds a single row of primary sporogenous cells. By anticlinal as well as periclinal divisions, the primary parietal layer produces two concentric layers, the inner being the tapetum, surrounded by the endothecium and the epidermis. The "middle layer", common in many anthers, is absent in this species.

The epidermis arises from the single outer layer of cells which only divides anticlinally. The outer wall of each epidermal cell has several projections similar to those on the surface of the aerial leaves. The cells of the epidermis at maturity are large and of even size, except at the region of dehiscence where they are smaller.

The layer beneath the epidermis, the endothecium, at maturity consists of radially elongated cells. At the "points" of dehiscence the inner walls of these cells produce several bands of thickening which run from the inner wall around each cell. The outer wall remains thin so that each cell when glanced at resembles a group of cells from the annulus of a fern sporangium. At dehiscence the contraction of the outer walls splits the pollen sac. The epidermal cells outside these thickened cells disintegrate early, perhaps because they are cut off by the thickened walls of the endothecium. In some anthers examined, thickening occurred on the walls of each cell of the endothecium.

The tapetum in *Zizania* is not greatly developed. It consists of radially elongated cells which divide by normal mitosis so that the uninucleate cells at maturity are the same size as the endothecium. The tapetum layer is still present when the pollen is shed. In *Oryza*, on the other hand, some tapetal cells are binucleate and they "degenerate and persist as a black strip surrounding the mass of microspores long before anthesis" (16).

In the four lobes of the anther each cell of the single vertical row of archesporial cells divides to produce two, then four, and later six or eight cells. These are wedge-shaped in a horizontal plane and are the spore mother cells. Each of these enlarges, divides by meiosis, and rounds up to form four daughter cells. The tetrads are isobilateral, or less frequently T-shaped or linear. The developing pollen grains thus form a single layer lining the microsporangium. Each is attached to the tapetum layer by a pore which remains prominent when the pollen grain is mature. The nucleus of the microspore divides to produce two nuclei. The generative nucleus appears elongate and smaller than the round central vegetative nucleus.

The mature microspore has a thick, smooth-walled exine devoid of markings as can be seen in Fig. 10, which is a picture of a germinating pollen grain. The outer surface of the intine is smooth while the inner surface appears

sculptured with coarse granulations. Within is dense cytoplasm containing the two nuclei and a vacuole (prominent in the developing microspore). The single pore which acts as a connection with the tapetum during development is circular in outline and in section is reminiscent of a bordered pit.

The Development of the Ovule

The walls of the ovary and the primordium of the ovule develop from the apex of the spikelet. The ovule is cauline in origin. The first sign of differentiation is the crescentic ridge which is formed on the side of the apex nearest the lemma. While the cells at the tip multiply to form the nucellus, the cells of the ridge multiply even more rapidly in a lateral as well as a vertical plane, to form the walls of the ovary, which completely encircles the nucellus. The ovary wall grows at an uneven rate, so that the developing ovule, which was initiated basally, assumes a lateral position (Fig. 4, *d*, *e*, and *f*). While this change is taking place, first one, then two annular outgrowths develop at the base of the nucellus, forming the inner and the outer integuments respectively.

As growth of the nucellus and integuments continues, the abaxial side of the placental region continues to grow until the ovule is in an anatropous position and attached directly to the posterior wall (the lemma side). The inner integument grows down until it surrounds the nucellus. In Fig. 13, an ovule is shown. In this section the tips of the inner integument are some cells apart as this is cut through the micropyle. The outer integument does not form a complete outer layer and disintegrates before the formation of the seed is complete. On the left side of Fig. 12, a slanting white line indicates the tip of the outer integument at its maximum growth. The single ovule is large and broadly elliptical in outline, nearly filling the cavity of the ovary. It is supplied by a large placental vascular strand which traverses the posterior wall of the ovary. It terminates in the chalazal region of the ovule quite close to the antipodal region of the embryo sac.

The ovary wall varies in thickness from five or six cells along the side to a thicker tissue at the top. The cells of epidermis and hypodermis (one to two rows) are all short and rectangular in cross section. The bulk of the wall, resulting from periclinal divisions, consists of isodiametric parenchyma cells. The cells of the inner epidermis are elongate, tabular cells similar to those of the outer epidermis, except that they divide less frequently and have elongate nuclei and dense cytoplasm. The short style at the upper end of the ovary produces two branches which bear papillae. Pollen grains caught on these papillae germinate and grow between the loose cells of the stylar canal towards the ovule. Figure 10 shows a germinating grain caught on these papillae at anthesis; the stylar arms within the palea and lemma grow out and downwards on each side of the palea.

Towards the top of the ovule, the outer integument is thickened broadly to form a wedge-shaped mass which projects into the base of the style (Fig. 4, *g*). True encountered this type of structure in *Zea mays* (34) and he suggested

that this structure may aid in determining the course of the descending pollen tube. At the micropylar end the outer integument may be two or three cells thick.

The inner integument is two cells thick except in the region of the micropyle where it forms an expansion which may be three or four cells thick. In this region the integument is in close contact with the cells of the nucellus. The integuments are completely free and show no fusion except at their points of origin.

The nucellus consists of large parenchyma cells, bounded by a distinct cutinized epidermis. The archesporial cell in the second layer of the nucellus becomes larger and more conspicuous than the surrounding cells (Fig. 5, *a*). No parietal cells are formed, so that the ovule may be described as tenuinucellate. Sometimes, however, the outer layer of the nucellus may divide periclinally, which may give the impression that parietal cells are formed (Fig. 5, *b*). The enlarged archesporial cell functions as the megaspore mother cell, undergoing meiotic division when it is about three times its original size, and producing a tetrad of cells (Fig. 5). Usually this tetrad is linear as shown in Fig. 5, *e*, but at other times, as in Fig. 5, *c* and *d*, it is T-shaped. Three of the cells in the tetrad disintegrate, while the fourth, usually the cell at the chalazal end, enlarges to become the embryo sac. Sometimes, the second megaspore from the chalazal end becomes functional.

Development of the monosporic embryo sac is of the 'normal' or 'Polygonum' type (18). The nucleus of the functioning megaspore divides to produce two, four, then eight nuclei, three at the micropylar end, three at the antipodal end, and two in the center of the embryo sac. The three cells at the micropylar end form the egg apparatus, the egg cell, and two synergids. At maturity, the

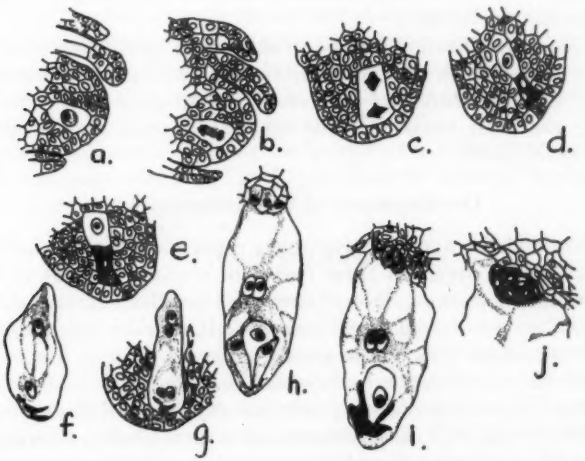

FIG. 5. Stages in the formation of megaspores and the development of the embryo sac.

synergids become indented, the upper part of each cell becoming the so-called 'filiform apparatus' (Fig. 5, *h*). The synergids start to disintegrate before fertilization, and stain deeply. The egg cell is a pear-shaped body with a prominent nucleus and a large vacuole (Fig. 5, *i*). The two polar nuclei in the center lie close to the egg apparatus and are connected to it by a conspicuous cytoplasmic strand. After fertilization these nuclei fuse to become the primary endosperm nucleus. The three antipodal cells divide rapidly to form a strongly developed tissue, consisting of from 6 to 20 cells in very close contact with the nearby nucellar cells. These are illustrated in Fig. 5, *j*, and stain darkly in Fig. 12. Their position in the chalazal region near the vascular supply of the ovule seems to ensure enough nutrient for their rapid development. In a recent study it has been found that *Zizania texana* also follows a normal type of development in the embryo sac (5).

Pollination and Fertilization

At anthesis, the lodicules of the pistillate spikelet swell, the cells becoming greatly distended. This swelling pushes the papillate stylar arms out from between the lemma and palea, leaving them exposed to the air and ready for pollination. A little more than an hour after the pollen grains are caught on the stigmatic hairs, they are found to have germinated (Fig. 10). The generative nucleus of each pollen grain divides to produce two sperm nuclei. Pollen tubes migrate through the loose cells of the stylar canal. They grow down between the ovary wall and the outer integument, and one finally enters the ovule through the micropyle. The rate of growth of the pollen tube is very rapid; within 2 hours after pollination it may be found in the embryo sac. After pollination, the stylar arms shrivel, and the cells of the lodicules become flaccid, cease their pressure, the palea returns within the margin of the lemma, and development continues.

At the time of fertilization, the two polar nuclei lie side by side and close to the egg cell. The pollen tube grows up through the micropyle and enters the embryo sac, the tip widens and is in close contact with both the cytoplasm surrounding the polar nuclei and the egg cell. Normal double fertilization apparently takes place.

Development of the Endosperm

At fertilization, the nuclear membranes separating the two polar nuclei disappear and the contents of these fuse with a sperm nucleus to form the primary endosperm nucleus. Seven hours after fertilization, its first division can be seen. The two triploid nuclei which result migrate to opposite ends of the embryo sac where they divide several times to produce free endosperm nuclei, scattered throughout the embryo sac. Subsequent divisions follow with great rapidity, all the nuclei dividing rhythmically. Figure 11 shows a developing endosperm in which all the nuclei are in a state of nuclear division. At this stage the embryo sac has enlarged by vacuolation and the endosperm nuclei are dispersed along the periphery of the embryo sac. At first the single nuclei

are separated by a thin lining layer of cytoplasm, but free nuclear division continues with such rapidity that the endosperm nuclei form a complete layer around the walls of the embryo sac, with very little cytoplasm. At times, the rate of nuclear division is faster than the rate of sac enlargement and the nuclei appear in groups all along the side. After many repeated divisions, these groups of nuclei come together to form a second lining layer of cells. At times the second lining layer is formed by the division of the outer layer of cells on one side only.

About the time that the parietal nuclei divide periclinally cytokinesis occurs. Cell walls are laid down, first in the antipodal region and later at the micropylar end. When the walls are first laid down, several nuclei are enclosed in a cell, but later new walls make them uninucleate.

By repeated cell division on one side only, the cells are produced on the inner side, until the central cavity of the endosperm is filled. This division of the cells on one side only was referred to by Gordon (11) in his work on the development of the endosperm of wheat, barley, and oats. He referred to the cambial activity of the lining layer of cells, which produced daughter cells on one side only. The same occurrence was referred to by Anderson (1) in her description of the caryopsis of *Poa pratensis* and *Poa compressa*.

The antipodal cells appear in the upper center of Fig. 12. They appear at the end of the recenty fertilized embryo sac. However, the enlargement of the embryo sac and the rapid growth of the endosperm causes the antipodal cells to assume a lateral position within the mature embryo sac. At maturity practically all the tissue of the nucellus has been absorbed by the developing endosperm, and the integuments have largely disintegrated leaving only a thin pericarp. In the mature caryopsis the endosperm consists of large thin-walled cells packed with large starch grains and with minute intercellular spaces at the corners. The two outer layers instead of being starchy are the aleurone layers, which stain deeply with safranin. Sometimes the distal portion of the endosperm produces a lobe which bends over to form a fold on the germinal face of the caryopsis. In section, this fold appears as an isolated "island" of endosperm tissue. This has been noted in *Zea* (27).

The embryo becomes completely enclosed by the endosperm as a development from the stage shown in Fig. 11, but later it becomes displaced to one side surrounded by partly digested cells.

Embryogenesis

The embryogeny of *Zizania aquatica* is a slight modification of the *Poa* variation of the Asterad type. Using the letter designations originally employed by Soueges the early stages of embryogenesis can readily be traced (15).

In Fig. 6, *a*, the zygote is shown before cell division begins. The first division is transverse as shown in Fig. 6, *b*, dividing the zygote into an upper terminal cell designated *ca*, and a lower cell designated *cb*. The upper cell *ca* is then divided by a vertical wall to give two daughter cells *q* (Fig. 6, *c*). This is followed by a horizontal division of the basal cell *cb*. The upper cell is designated *m*

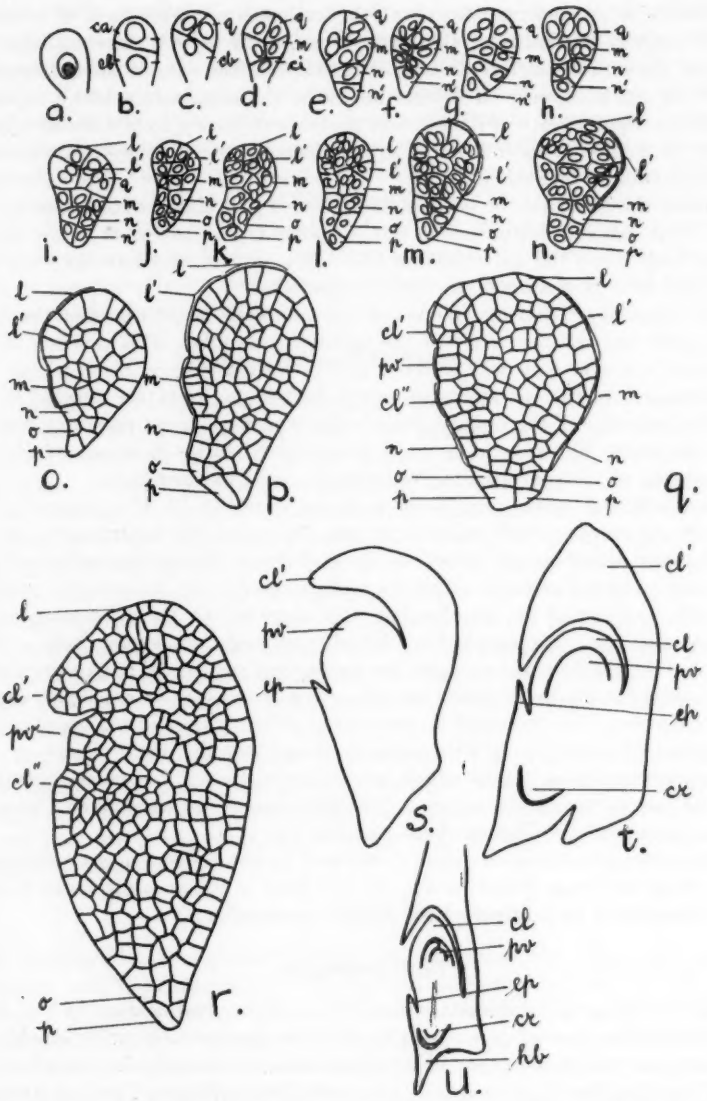

FIG. 6. Development of the embryo. Scale: *s* and *t*, 1/5 of that of *a–r*; *u*, 1/15 of that of *a–r*.

cl', upper and lower tips of the scutellum; *cl*, coleoptile; *cr*, coleorhiza; *ep*, epiblast; *hb*, hypoblast; *pv*, stem apex and first leaf.

and the lower one *ci* (Fig. 6, *d*). At this stage the development differs slightly from that of *Poa*, where *cb* divides first, followed by *ca*.

The cells *m* and *ci* divide simultaneously, *m* by a vertical crosswall and *ci* by a horizontal wall to form two tiers *n* and *n'* as shown in Fig. 6, *e*.

Both tiers *n* and *n'* are further divided by vertical walls (Fig. 6, *i*). Tier *n'* shows some difference from the normal *Poa* variation. It is divided by a vertical wall into two cells one of which is again divided by a transverse wall to give rise to two tiers *o* and *p* (Fig. 6, *j* and *k*). The tier *p* forms a short suspensor.

The tier of cells labelled *q* is divided vertically and later transversely to form the layers *l* and *l'*. Figure 6, *i*, shows an embryo in which a transverse wall has been laid down on one side but not on the other. In Fig. 6, *j* and *n*, *l* and *l'*, can be seen in stages of further subdivisions.

By the stage illustrated in Fig. 6, *n*, the embryo has begun to show some lateral development. Bulges become apparent on one side of the embryo and not the other. An upper bulge can be distinguished in the *l* and *l'* region of the embryo and a lower bulge in the *m* region. The depression between these two bulges is at the junction of the *l'* and the *m* tiers (as in *Poa*). The upper bulge formed from *l* and *l'* is designated *cl'* and forms the upper part of the scutellum (Fig. 6, *r* and *s*).

In Fig. 6, *q*, a third bulge *pv* has developed between the upper and lower ones. This third bulge later develops into the stem apex and first-formed leaves, and the third bulge is the lower part of the scutellum which later becomes the epiblast. Figure 6, *t*, shows a developing embryo in which the bulge *pv* has differentiated to form the coleoptile and primary leaves. The bulge *cl'* has become the upper part of the scutellum while *cl* has not differentiated much further and has become the epiblast. In *Poa*, the coleoptile is formed from *cl* as well as *cl'*. The tier *p* divides to produce the suspensor while the rest of the embryonic tissue becomes the mesocotyl and the radicle surrounded by the coleorhiza.

Extensive growth occurs in a vertical direction so that the embryo changes from an almost circular outline to markedly elongated structure. In its course of development the embryo increases its length approximately 100 times, but its width only about 5 times. The later stages in the embryogenesis have already been described (17).

In the mature embryo the apex is wrapped in three or four leaf primordia and surrounded by the coleoptile. The apex is smaller than in an actively growing plant, being 66 μ in length and 45 μ in width, cf. 97×103.

The Structure of the Mature Caryopsis

The mature caryopsis is cylindrical with tapering ends and is approximately 16 millimeters in length, and 1.5 millimeters in diameter. Figure 8 shows spikelets with fully matured seeds completely surrounded by a paper-thin palea and lemma, which have already been described. The testa of the seed is dark brown, and the endosperm within is almost colorless. The remnants of the

stylar projection are visible at the tip (Fig. 1, *c*, *d*, and *e*). From this point a ridge runs all the way down one side of the grain to its base. This ridge is made by the placental vascular supply. The larger ridge on the opposite side is due to the embryo (Fig. 1, *e*). The part of the embryo exposed is yellow or yellowish green, fairly flat, and flush with the seed surface, with a characteristic arrowheaded shape, and is approximately 9 millimeters long. It is free from the grain except at the base where it is attached to the scutellum. The scutellum makes contact with the endosperm along the whole of its length. In Fig. 1, *e*, a small spear of tissue, the epiblast, can be seen lying on the lower surface of the embryo. This thin, white, non-vascular outgrowth of tissue extends from the base (the point of attachment of the scutellum) up to the arrowhead-like swelling.

The anatomical structure of the caryopsis and its significance have been dealt with elsewhere (26, 24). The germination and seedling characteristics have also been described (2, 26).

Discussion

The purpose of this study has been to describe the stages in the growth and development of *Zizania*. However, the detailed study of these processes reveals some data which have bearing on problems related to morphology and taxonomy (32). In studying the inflorescence of *Zizania*, considerable emphasis has been placed on the affinities of the organs with those of other plants. An attempt will be made to answer some questions arising from this study.

1. What interpretation should be given to the two structures at the base of each spikelet (labelled *cl* and *vg* in Fig. 4)?

As shown earlier, these structures are differentiated first on each spikelet. This suggests that these outgrowths are the remains of important organs.

In her discussion of the morphology of rice, *Oryza sativa* L., Arber (2) noted that the one-flowered spikelet consists of a lemma and palea, preceded by two glumes which are often interpreted as 'outer empty glumes'. Below these are two small structures regarded by some authors merely as 'cupular outgrowths', and by others as an expansion of the pedicel. Arber disagrees with this, and by analogy with the grass *Anthoxanthum* she names the parts of the rice flower as follows: the two structures at the base she calls the outer empty

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FIG. 7. Inflorescence of *Z. aquatica* showing staminate spikelets below and pistillate spikelets above. ×0.2

FIG. 8. Branch of inflorescence with three spikelets, the lowest a hermaphrodite with well-developed stamens and pistil. ×1.3

FIG. 9. Longitudinal section of young inflorescence with spikelet initials at the tip of each branch. The inflorescence is surrounded by numerous multicellular hairs. ×40

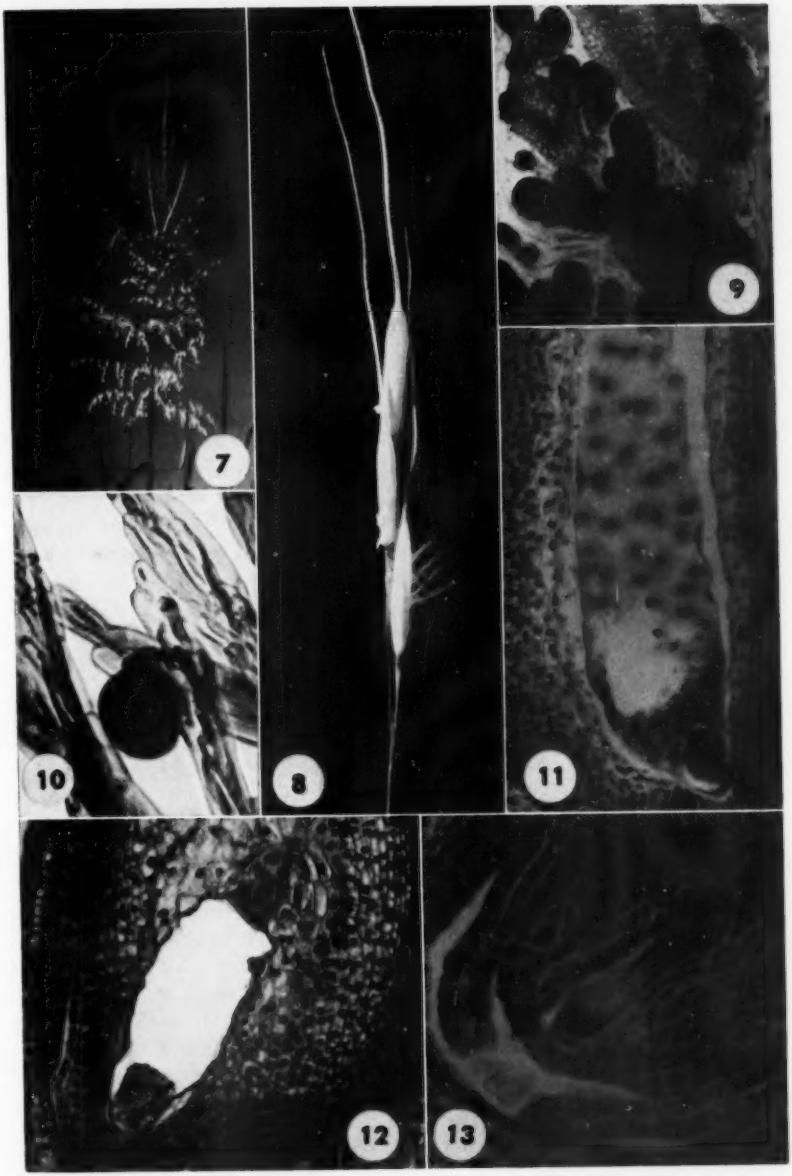
FIG. 10. Germinating pollen grain on stigmatic hairs. ×100

FIG. 11. Longitudinal section of ovary showing numerous endosperm nuclei in metaphase. At the bottom of figure the developing embryo is seen surrounded by dense cytoplasm and many nuclei. ×125

FIG. 12. Longitudinal section of ovary after fertilization with developing embryo and remains of synergids at the micropylar end. A free endosperm nucleus is on the right of the sac while a group of darkly stained antipodals appears at the end. As the sac enlarges it grows beyond these in the region of the lightly stained cells. ×150

FIG. 13. Longitudinal section of young ovule showing four-nucleate embryo sac. ×250

PLATE I



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glumes (in an extremely reduced form), the two bracts succeeding them she calls the sterile lemmas, and the upper two, the lemma and palea. In *Luziola*, Arber (2) also found vestigial, non-vascular outer glumes. In the light of this, the morphology of the spikelet of *Zizania* can be interpreted as follows: the lower extension which is supplied with vascular strands is the outer empty glumes, while the callosity is a reduced non-vascular form of the two sterile lemmas.

2. What interpretation should be given the two bracts surrounding each spikelet?

The two bracts surrounding the one-flowered spikelet of *Zizania* (lemma and palea) have received varied interpretations. Most workers use the name lemma and palea as suggested by Hackel (12), but Bentham and Hooker (4) called both bracts glumes without making any distinctions. Hitchcock (14), however, treats them as lemma and palea in the pistillate spikelet, and as the second glume and lemma in the staminate spikelet. Chase (8) found it difficult to come to a definite conclusion about these two bracts. She regards them as lemma and palea in both the pistillate and staminate spikelets, but characterizes the palea as anomalous in having three nerves. She later found this solution unsatisfactory and came to the alternative conclusion that the two bracts may be the first glume and the lemma, the second glume and the palea being absent.

Weatherwax (35) finds that any doubt as to the identity of the three-nerved bract in the staminate spikelet should apply equally well to the three-nerved bract in the pistillate spikelet, since there is no significant anatomical difference, both having a midrib, and developing in the same manner. Weatherwax (35) concludes that the bracts are both lemma and palea in the staminate as well as the pistillate spikelets, because, in order to say as Hitchcock does (13) that the three-nerved bract is the lemma, it must be assumed that the flower has rotated through an angle of 180°, to account for the normal position of the ovule and the lodicules. The observations from this work reveal nothing to make the stand taken by Weatherwax concerning the lemma and palea untenable.

3. Does the vascular supply to the ovule shed any light on the theory of the three-carpellary nature of the ovule of grasses?

Transverse sections through the developing ovule show the presence of three vascular strands; two persist and enter the stylar arms, while the third, to the anterior side of the ovule, disintegrates quite early in the development of the ovule. In some ovules, this strand was not visible. However, in general the presence of three strands supports the convention that the unilocular ovary consists of three carpels. The placental vascular supply develops later at the posterior end of the ovule after the anterior strand has disappeared. This placental strand can therefore be considered the fused marginal veins of the lateral carpels.

4. Is the coleoptile in the grass embryo the homologue of a leaf, or is it part of the cotyledon (scutellum)?

In studying the embryogenesis of *Zizania*, it was found that the coleoptile developed in a manner similar to the primary leaves from the central hump of tissue seen in Fig. 6, *r* and *s*. This is a modification of the *Poa* variation, where the coleoptile develops as part of the scutellum (15). Although there are only two vascular strands supplying the coleoptile, it is possible that the third one has become so reduced as to be non-existent. In support of the foliar nature of the coleoptile, reference must be made to the structure of the coleoptile of *Jouvea pilosa* (Presl.) Scribn. (23) in which three vascular strands have been demonstrated in the coleoptile, as well as one in the scutellum. The coleoptile here has a midrib, hence it can be called the first-formed leaf of the embryo.

Further evidence that supports the foliar nature of the coleoptile can be found in the work of La Rue and Avery (17) on the embryo of *Zizania aquatica*. When excised embryos were grown in culture solutions, growth of the cotyledon was retarded, while the coleoptile and shoot showed precocious development, increasing greatly in length. This behavior of the coleoptile suggests a greater affinity with the leaves than with the cotyledon.

5. What evidence is there that *Zizania* and *Oryza* are related?

Although the chromosome number in these two genera indicates little affinity, Reeder (24) from his study of the embryo has concluded that these genera are closely related. The very close similarity in the anatomy of the emergent leaves of *Zizania* with those described from *Oryza* suggests an affinity, as do many other anatomical features. However, the persistent tapetum layer in the pollen sacs suggests a divergence.

Acknowledgments

The authors would like to express their gratitude for a grant from the Ontario Research Foundation. The Division of Research, Department of Lands and Forests, Ontario, supplied seed and information on the distribution of *Zizania* in Ontario. Dr. W. G. Dore, Plant Research Institute, Science Service, Ottawa, has given sympathetic assistance in the study. Appreciation is also due to Professor R. T. Riddell of the Botany Department, Ontario Agricultural College, whose advice was particularly valuable in the study of embryogenesis.

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INFLUENCE OF MATERNAL TISSUE ON LOOSE SMUT INFECTION OF HYBRID BARLEY KERNELS[1]

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Abstract

The influence of maternal tissues on loose smut (*Ustilago nuda* (Jens.) Rostr.) infection of hybrid tissues was investigated through histological studies of artificially inoculated kernels of susceptible and resistant varieties of barley and their reciprocal F_1 hybrids. Depending on the stage of development of the kernel scanty to abundant mycelium was observed in the crease region, integument, aleurone, endosperm, and embryo of the susceptible kernels. In kernels of the resistant varieties, mycelium was not observed in the aleurone, endosperm, or embryo although present in the chalazal region and the parenchyma associated with the vascular bundle. After floret inoculation, the maternal tissues of F_1 hybrid kernels of reciprocal crosses between the susceptible and resistant varieties had a mycelial development similar to that in the female parental varieties. Hybrid tissues of Odessa (susceptible) × Anoidium possessed a scanty mycelial development, whereas those of the reciprocal appeared to be free. Hybrid tissues of the reciprocal crosses between Odessa and Trebi had a scanty mycelial development. These observations indicated that maternal tissues influenced to some extent infection of the hybrid embryo. However, this influence appeared to be secondary in importance to that of the genotype of the embryo itself.

Introduction

Loose smut of barley, incited by *Ustilago nuda* (Jens.) Rostr., establishes itself in the young developing kernel and is present as dormant mycelium in the mature caryopsis.

Little is known of the exact nature of resistance to loose smut of barley. Inheritance studies indicate that resistance is incompletely dominant or dominant (11). Therefore the F_1 of a cross between a susceptible and a resistant variety, and of its reciprocal, should be resistant since the embryos have the same genotype. Several investigators have questioned whether the smut reaction of hybrids depends on the genetic make-up of the embryo or on the influence of the surrounding maternal tissue. Most of them (5, 7, 13, 14, 17) came to the conclusion that the smut reaction of hybrids depends on the genetic constitution of the embryo. Shands (16) obtained results with certain hybrids indicating that resistance is located partly in the maternal tissues surrounding the embryo.

Conceivably, resistant maternal tissue might prevent or at least delay growth of the loose smut fungus toward the hybrid embryo and allow it to escape infection. Susceptible maternal tissue that offers no barrier to the growth of the fungus may expose the embryo to infection.

[1]Manuscript received April 11, 1960.
Joint contribution from the Department of Agronomy, University of Wisconsin, Madison, Wisconsin, U.S.A.; the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture; and the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa (contribution No. 30). Part of a thesis submitted to the Graduate School, University of Wisconsin, in partial fulfillment of the requirements for the Ph.D. degree.
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A study was conducted to obtain information on mycelial development in maternal tissues as related to subsequent infection of hybrid tissues. Kernels developed after inoculation of florets of susceptible and resistant varieties and their reciprocal F_1 hybrids were studied histologically.

Materials and Methods

One susceptible and two resistant varieties and the reciprocal crosses between the susceptible and the resistant varieties were studied. The loose smut reactions of the three varieties, when artificially inoculated with *U. nuda*, were as follows: Odessa, C.I. 934,[4] 92.3% infected plants; Anoidium, C.I. 7269, 6.5%; and Trebi, C.I. 936, 7.6%.

By use of the needle method (15), plants were inoculated 24 hours after pollination with the "common" race of *U. nuda* to which Trebi is resistant. Kernels were collected and fixed 12, 15, 20, 25, and 30 days after inoculation, for studies of the development of the fungus at different stages of growth of the host. Noninoculated kernels of the parent varieties were collected at similar stages.

The material was fixed with alcohol–acetic fixative (3 parts 100% ethyl alcohol and 1 part glacial acetic acid) as recommended by Dickson and Shands (3) and it was stored in 70% ethyl alcohol. Johansen's (4) suggestions were followed in dehydration, infiltration, and embedding. Serial microtome sections, 10 μ in thickness, were obtained by cutting longitudinally at right angles to the dorsoventral surfaces of the embedded kernels. A few kernels were cross-sectioned.

Sections were stained with a modified form of Conant's quadruple stain (4) in which thionin (Lauth's violet, C.I. No. 920[5]) was substituted for the crystal violet of the Conant's stain. Another staining method used to some extent involved a combination of iodine-ripened hematoxylin (1), safranin, and fast green. Conant's stain, as modified, gave slightly better differentiation than did the hematoxylin stain.

Results

Observations of Parents

Development of loose smut mycelium in tissues of the pistil in the varieties Odessa, Anoidium, Trebi, and their F_1 hybrids was observed.

Spores of *U. nuda* lodged on the stigma, style, and ovary wall of the inoculated florets where they germinated and penetrated the host. These three principal regions of penetration were observed in Odessa (susceptible). A single pistil may be invaded in all three regions.

Growth of the mycelium in the interior of the style was limited principally to the conducting tissue (Fig. 1). The mycelium subsequently reached the distal end of the ovary. It proceeded farther, crossing the cross cells of the

[4]C.I. refers to accession number of the Cereal Crops Research Branch, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture. The Odessa used here was a strain originating from C.I. 934.

[5]C.I. No. refers to the Colour Index of the Society of Dyers and Colourists.

ovary[6] (Fig. 2). There was abundant growth in the distal end of the kernel where the mycelium invaded the integument[7] and the aleurone cells. The fungus grew intra- and inter-cellularly (Fig. 3). The endosperm cells did not appear to be invaded at this stage (Fig. 4). The mycelium proceeded toward the proximal end of the kernel, growing mainly in the integument (Fig. 6).

In another mode of penetration, the loose smut fungus, instead of penetrating the integument directly, grew in the direction of the chalaza, became established in the parenchyma (Fig. 7) associated with the vascular bundle, and spread to the nutrient tissue[8] and to the pericarp (Fig. 8). From the chalaza, the mycelium passed to the integument (Fig. 9).

The mycelium may penetrate directly through the ovary wall (Fig. 10). It was observed in the epidermis of ovaries 15 days after inoculation (Figs. 11 and 12). Pericarp invasion may be relatively heavy under some conditions (Fig. 13). From the pericarp, the mycelium reached the integument by way of the cross cells (Fig. 14). The integument became heavily infected (Figs. 15 and 16).

At a later stage in the development of the fungus, the mycelium penetrated the partially disorganized cells of the endosperm adjacent to the scutellum of the embryo (Fig. 17). Finally, the scutellum itself was invaded through the epithelial cells in contact with the partially disorganized cell layer of the endosperm (Fig. 18). The mycelium developed subsequently in most tissues of the embryo.

Kernels of Odessa, 12 days after inoculation, contained abundant mycelium in the integument. Mycelium was moderate in the crease[9] region and scanty in the aleurone and embryo (Fig. 19). It was abundant in the embryo 25 days after inoculation. No mycelium was observed in most of the inoculated kernels of Anoidium and Trebi. In those in which it was found, its development was moderate to scanty in Anoidium and scanty in Trebi, in the crease region and in the integument on the ventral side of the kernel. It was absent in the aleurone and embryo. When mycelium was found in Anoidium, it was only in kernels collected 12 or 15 days after inoculation.

Observations of F_1 Hybrids

Mycelium was moderate to abundant in the crease region, abundant in the integument, and scanty in the aleurone and embryo of Odessa × Anoidium F_1 kernels 12 days after inoculation. It was present in the distal end (Fig. 5) as well as in the proximal end of the kernel. In Fig. 20 the fungus crossed the aleurone layer and penetrated the partially disorganized cells of the endosperm

[6]"Cross cells" refer to the thick-walled cells adjacent to the inner epidermis of the pericarp (18). Since the inner epidermis of the pericarp was mostly absorbed by the seventh day of kernel development (18), the cross cells were adjacent to the integument in the material observed.

[7]"Integument", as used here, refers to the inner integument. The outer integument, having disintegrated by the fifth day of kernel development (18), was not visible in the material observed.

[8]"Nutrient tissue" (sheaf cells of Collins) refers to the sheaf-like mass of elongated cells radiating from the groups of cells of glandular character with somewhat thickened walls lying immediately between the points of origin of the integument (2, 10).

[9]The "crease" included the pericarp, integument, and vascular bundle associated with the chalaza on the ventral side of the kernel.

adjacent to the scutellum. Mycelium was scanty in the aleurone, the embryo, and the partially disorganized cells of the endosperm adjacent to the scutellum 20 and more days after inoculation. Kernels of the reciprocal cross contained moderate to abundant mycelium in the crease region (Fig. 21) and in the integument 12 days after inoculation, but none was found in the aleurone or embryo. It was not observed in older material.

Mycelium was moderate to abundant 12 days after inoculation in the crease region and the integument (Figs. 22 and 23) and scanty in the aleurone and embryo (Fig. 22) of Odessa × Trebi F_1 kernels. It was scanty to moderate in embryos 15 and more days after inoculation. Kernels of the reciprocal contained scanty to moderate mycelium in the crease region and the integument (Fig. 24) 12 or 15 days after inoculation, but none in the aleurone and embryo. Mycelium was scanty in the aleurone and embryo 30 days after inoculation.

Discussion

In general the observations made in this study agree with those of Lang (6) and Ruttle (12). Lang found two modes of penetration and development of the loose smut fungus in inoculated barley kernels: (1) through stigma, style, and chalaza; and (2) directly through the ovary wall. In addition he suggested penetration through stigma, style, inner epidermis of pericarp, and integument at distal end of the kernel. He was unable to observe mycelium in the integument at the distal end of the kernel and concluded that mycelium is unlikely to follow the latter mode of development. In the present investigation, mycelium was found on many occasions in the cross cells and the integument at the distal end of the very susceptible Odessa kernels.

A number of investigators have found mycelium in the embryo of infected kernels of susceptible varieties of wheat and barley (9). Embryos of inoculated kernels of susceptible Odessa contained abundant mycelium, while those of resistant Anoidium and Trebi contained none. Ruttle (12) reported similar results in one inoculated resistant variety. Popp (9) found mycelium in a few embryos of Trebi kernels inoculated with a race of *U. nuda* to which it is resistant. The larger number of kernels examined by him may account for the difference between his results and those reported here.

The presence of mycelium in maternal tissues of Anoidium kernels only in the earlier stages of host development may be due to a build-up of the incompatibility of host and parasite, as suggested by Oort (8) in wheat. The finding of mycelium in the maternal tissues of Trebi kernels agrees with the observations of Ruttle (12), who found mycelium in the pericarp of resistant wheat and barley after floral inoculations.

In Odessa × Anoidium, the mycelium developed abundantly in the susceptible maternal tissues. It was scanty in the embryo of the hybrid and abundant in that of the Odessa parent. Its rapid growth in the susceptible maternal tissues was a factor of importance in increasing the chance of invasion of the hybrid embryo. No mycelium was observed in the embryo or in any other hybrid tissue of the reciprocal cross. Embryos of both crosses have the

same genotype. The situation encountered in the reciprocals suggested some influence of the maternal tissues on mycelium reaching the embryo. However, since embryo infection was scanty in Odessa × Anoidium, it was unlikely that the F_1 plant grown from it would produce smutted heads. Therefore the reciprocal crosses may show the same degree of loose smut resistance. This conclusion is well supported by that of Livingston (7), Schaller (13, 14), and Skoropad and Johnson (17), who observed F_1 plants rather than F_1 embryos.

Popp (9) reported a close relation between the incidence of mycelium in embryos and the incidence of smut in adult barley plants. It is questionable whether Popp's method for determining mycelium could have detected the scanty mycelial development observed in stained microtome sections of Odessa × Anoidium embryos. Moreover, the scanty mycelium present probably was not enough to invade the developing seedling and infect the F_1 plant, although no experimental evidence was obtained on this point.

About the same degree of embryo infection was observed in older F_1 kernels of the reciprocal crosses between Odessa and Trebi. This slight infection of the embryo was unlikely to produce smutted heads on F_1 plants.

Observations on hybrids and parents in this study show that the maternal tissue definitely influences infection of the hybrid embryo. However, this influence is second in importance to that of the embryo itself.

Acknowledgments

The authors are indebted to Emma Fisk and J. G. Dickson for aid in the interpretation of histological structures of the barley caryopsis and to Eugene Herrling for the photographs. The senior author is grateful to the Agricultural Research Council of the Province of Quebec for financial assistance.

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EXPLANATION OF FIGURES

FIGS. 1 to 4. Mycelium of *U. nuda* in Odessa kernel, 12 days after inoculation.

FIG. 1. Mycelium (M) in conducting tissue (C) at base of one of the two styles (S). ×398

FIG. 2. Mycelium (M) in cross cells (C) at distal end of kernel. Integument (I), remains of nucellus (N), aleurone (A). ×350

FIG. 3. Mycelium (M) in aleurone (A) at distal end of kernel. Cross cells (C). ×398

FIG. 4. Mycelium (M) in integument (I), and aleurone (A) at distal end of kernel. Cross cells (C), endosperm (E). ×398

FIG. 5. Mycelium in Odessa × Anoidium F_1 kernel, 12 days after inoculation. Mycelium (M) in integument (I), and in cross cells (C) of pericarp (P) at distal end of kernel. Aleurone (A), endosperm (E). ×398

FIG. 6. Mycelium in Odessa kernel, 12 days after inoculation. Mycelium (M) across two cells of the integument (I). Aleurone (A), endosperm (E), pericarp (P). ×398

FIGS. 7 to 9. Mycelium of *U. nuda* in Odessa kernel.

FIG. 7. Mycelium (M) in chalaza (CH) and parenchyma (P), 15 days after inoculation. Xylem (X). ×398

FIG. 8. Mycelium (M) in pericarp (P), chalazal region (CH), and nutrient tissue (N) at distal end of crease region, 12 days after inoculation. ×358

FIG. 9. Mycelium (M) in chalazal region (CH), and integument (I), 12 days after inoculation. Pericarp (P), nutrient tissue (N). ×358

FIG. 10. Mycelium in Odessa × Anoidium F_1 kernel, 15 days after inoculation. Mycelium (M) across pericarp (P). ×368

FIGS. 11 and 12. Mycelium in Odessa kernels, 15 days after inoculation. Mycelium (M) in epidermis (EP) of pericarp and inner pericarp tissue (P) in crease region. ×398

FIGS. 13 to 18. Mycelium of *U. nuda* in Odessa kernel.

FIG. 13. Mycelium in pericarp at proximal end of kernel, in crease region, 15 days after inoculation. ×398

FIG. 14. Mycelium (M) in cross cells (C) and integument (I) in crease region, 12 days after inoculation. Pericarp (P). ×398

FIG. 15. Mycelium (M) in integument (I) at proximal end of kernel, 12 days after inoculation. Dorsal side. Cross cells (C), pericarp (P), endosperm (E). ×350

FIG. 16. Mycelium (M) in integument (I), and aleurone (A) at proximal end of kernel, 15 days after inoculation. Cross cells (C), pericarp (P). ×398

FIG. 17. Mycelium (M) in the partially disorganized cell layer (L) of endosperm (E) adjacent to the scutellum of the embryo (EM), 15 days after inoculation. ×398

FIG. 18. Mycelium (M) in epithelial cells (EP) of scutellum (SC), and other cells of scutellum, 30 days after inoculation. Partially disorganized layer of endosperm (L). ×378

FIG. 19. Mycelium of *U. nuda* in Odessa kernel, 12 days after inoculation. Mycelium (M) in embryo (EM) and integument (I). Cross cells (C). ×378

FIG. 20. Mycelium in Odessa × Anoidium F_1 kernel, 12 days after inoculation. Mycelium (M) in aleurone layer (A) and partially disorganized cells (L) of endosperm (E) adjacent to the scutellum (SC). Integument (I), pericarp (P). Proximal end of kernel. ×398

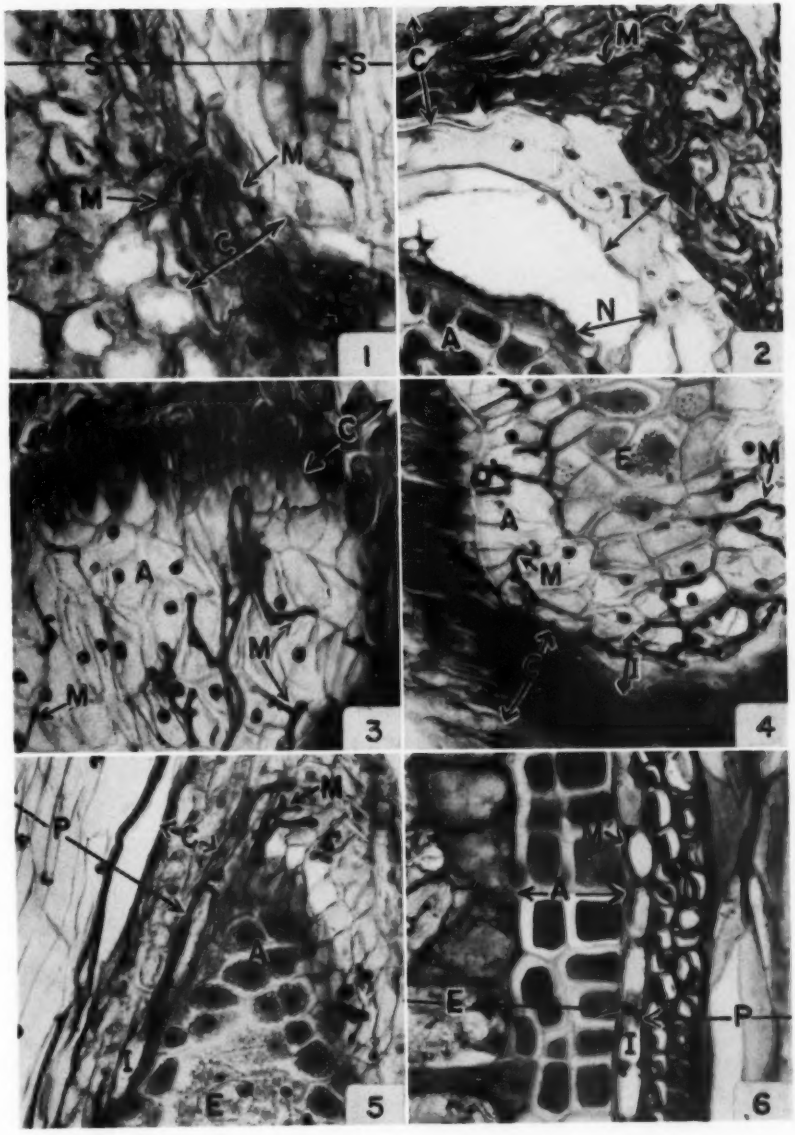
FIG. 21. Mycelium in Anoidium × Odessa F_1 kernel, 12 days after inoculation. Mycelium (M) in integument (I) in crease region. Cross cells (C), endosperm (E). ×378

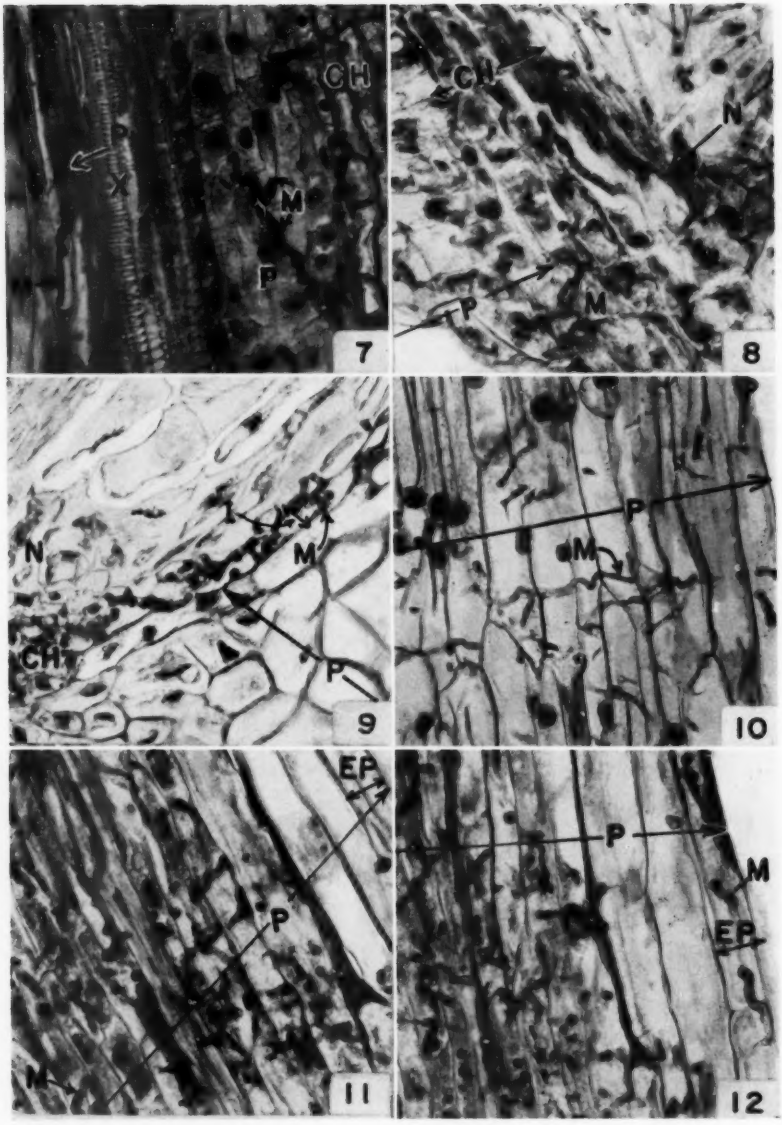
FIG. 22. Mycelium in Odessa × Trebi F_1 kernel, 12 days after inoculation. Mycelium (M) in integument (I), aleurone (A), and partially disorganized layer (L) of endosperm (E) adjacent to the scutellum. Mycelium is also in embryo (EM). ×378

FIG. 23. Mycelium in Odessa × Trebi F_1 kernel, 12 days after inoculation. Mycelium (M) in cross cells (C) and integument (I) at distal end of kernel. Aleurone (A). ×378

FIG. 24. Mycelium in Trebi × Odessa F_1 kernel, 15 days after inoculation. Mycelium (M) in integument in ventral side of kernel. Aleurone (A), endosperm (E), cross cells (C). ×378

PLATE I

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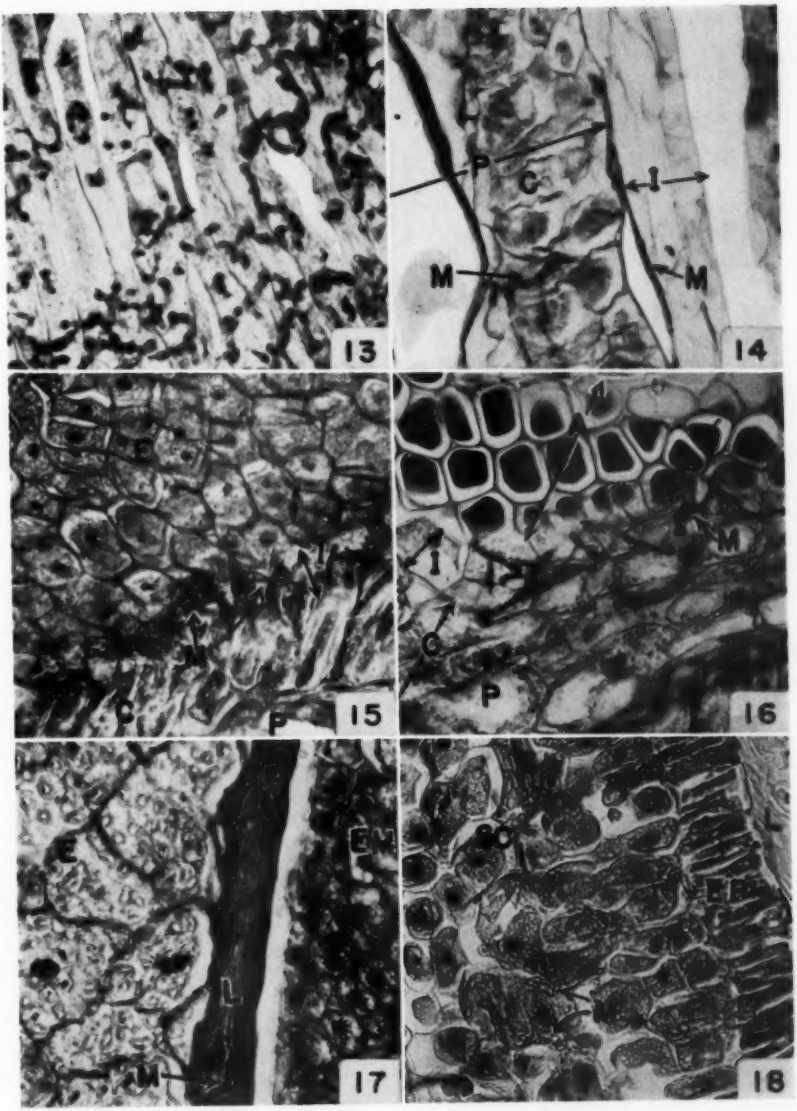
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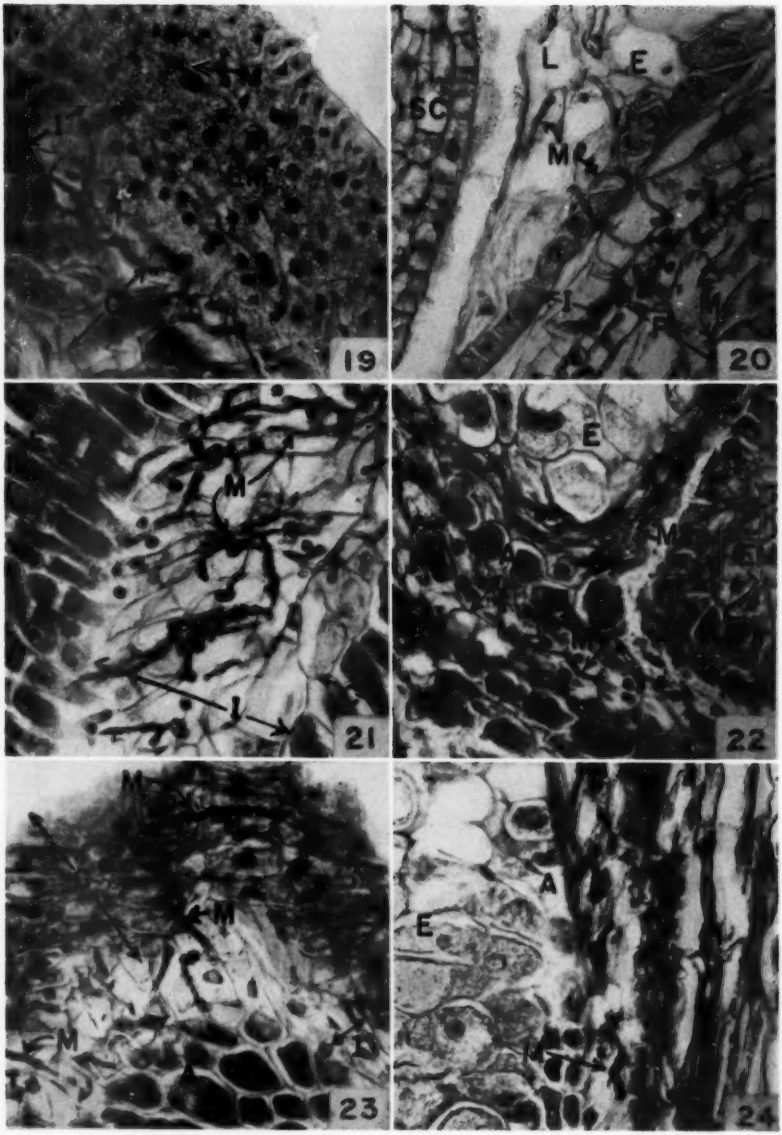
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CYTOTAXONOMY AND INFRASPECIFIC VARIATION OF AGROPYRON SMITHII RYDB.[1]

J. M. GILLETT AND H. A. SENN

Abstract

Agropyron smithii Rydb. is considered to be the correct name of the grass commonly known as western wheat grass. The pubescent variety *molle* has been found to have no distinct geographical distribution within the species and is therefore reduced to the rank of form. Many of the eastern localities recorded are attributed to introduction rather than to extension of the species range. Characters of pubescence and awning show relative uniformity within the clone but a much wider variation over the entire range. Spike, spikelet, and floret pattern is compared with those of related species. Somatic chromosome counts on 62 plants from points throughout the range indicate constant octoploidy ($2n=56$). Chromosomal rearrangements appear to be slight. The species does not cross with several other species tested and is apparently self-sterile.

Introduction

Agropyron and the companion genus *Elymus* are taxonomically difficult because of a high degree of variability in certain characters coupled with some natural hybridization of species within and between the genera. Considerable polyploidy on the same base number, particularly at the tetraploid level, exists.

In order to study species relationships and to delimit taxa in *Agropyron* it was considered useful to examine the nature of the variation of certain characters in *A. smithii*, a relatively stable species displaying some genetic isolation from the rest of the genus.

Materials and Methods

Live plants or seed have been gathered in the field at numerous places over a period of about ten years. Herbarium specimens have usually been collected as vouchers for this material. In addition, population samples have been gathered at a number of points in Canada and the northern United States. Most of these were random samples; in a few, straight-line transects were made. In the case of the latter, culms were taken every few yards until a representative number was obtained. A few samples were made by selection from apparent clones.

In the case of the hybrid and in collections for which chromosome counts are reported, the cytological accession number is given first. The collector and collection number, location, and other data follow in parentheses. By C following the accession number we mean that the material was grown from live plants collected in the field; and S indicates that the material was grown from seed.

[1]Manuscript received February 29, 1960.
Contribution No. 57 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

The type specimens of *A. smithii* and its synonyms have been examined. The validity of the name has been ascertained with respect to the International Code of Botanical Nomenclature. No change in name or of the choice of type has been made but an argument for the retention of the selection made by the original author is presented.

Plants obtained in the field from the same localities as the herbarium voucher specimens have been grown in pots both in the greenhouse and outside. Because of the rhizomatous nature of the species, pots are required to confine the cultures. Somatic chromosome numbers were obtained from root tips prepared from this source.

Results

Taxonomy

Western wheat grass was originally described as *Agropyron spicatum* by Scribner and Smith (1897), the name having appeared first as a *nomen nudum* (Scribner and Smith 1896). These authors included *Festuca spicata* Pursh (1814), *Triticum missuricum* Sprengel (1825), and *Agropyron glaucum occidentale* Vasey & Scribner (Macoun 1888) in synonymy. They also designated a type specimen as, "Type in the Engelmann herbarium collected by Geyer, 'Upper Missouri'."

Since they included *Festuca spicata* Pursh in synonymy, they were publishing a new combination, *Agropyron spicatum* (Pursh) Scribn. & Smith, rather than a new name, and according to Article 55 of the Code (1956), the new combination must be retained for the species to which the epithet was originally applied and must be attributed to the author who first published it.

Rydberg was the first to notice that Scribner and Smith had confused two species. He therefore renamed their species *Agropyron smithii* but unhappily cited *Agropyron spicatum* Scribn. & Smith in synonymy. However, he referred to his treatment of *A. spicatum* on a previous page. On this page (p. 61) he included *Festuca spicata* Pursh correctly in the synonymy of *A. spicatum* and thus excluded it from his *Agropyron smithii*. This exclusion of the type from *A. smithii* and from *A. spicatum* sensu Scribn. & Smith makes it possible to apply the epithet *smithii* to Scribner and Smith's species.

The two other synonyms included by Scribner and Smith now warrant discussion.

Triticum missuricum Spreng. (1825) was described as follows: "missuricum 19. Tr. spica elongata recta, spiculis alternis floris, flosculis acuminato-subulatis, foliis abbreivatis linearibus culmoque glaucis glabris. Ad. fl. Missur. (*Festuca spicata* Pursh)." Since *Festuca spicata* Pursh is included in synonymy, *Triticum missuricum* has the same type and is therefore synonymous with it.

The final item in Scribner and Smith's synonymy is *Agropyron glaucum* var. *occidentale*. Macoun (1888) attributed this variety to Vasey and Scribner. However, this variety was published by Scribner in 1885 (Scribner 1885).

We are obliged under Appendix IV (4*d*) of the Code (Art. 7, Note 4) to take up the type of the residue. The type of *A. smithii* then should be a Kellerman specimen examined by Scribner, i.e. the type of *A. glaucum* var.

occidentale Scribn. However, after a diligent search we have not been able to locate the type of this variety. Therefore we shall continue to recognize the selection of Geyer's specimen made by Scribner and Smith as the type of *A. smithii* Rydb.

To summarize, *A. spicatum* Scribn. & Sm. is based on *Festuca spicata* Pursh and hence must be retained for this species. *Triticum missuricum* Spreng. is synonymous with *A. spicatum*. The type of *A. glaucum* var. *occidentale* should become the type of *A. smithii* but since the Geyer specimen is the only specimen available it should be retained as the type.

The emended description with synonymy follows:

Agropyron smithii Rydb. in Mem. N.Y. Bot. Gard. 1: 64. Feb. 15, 1900. (Type: *Geyer s.n.* MO!; photo DAO.)

A. glaucum R. & S. var. *occidentale* Scribn. in Trans. Kans. Acad. 9: 119. 1885. (Type: unknown.)

A. occidentale Scribn. in U.S. Dept. Agr. Agrost. Circ. 27: 9. Dec. 4, 1900.

Zeia occidentalis (Scribn.) Lunell, Amer. Midl. Nat. 4: 226. 1915.

Agropyron spicatum Scribn. & Sm. var. *viride* Farwell in Mich. Acad. Sci. Rept. 21: 356. 1902. (Lectotype selected by Hitchcock, Man. Gr. U.S. 777. 1935: *Farwell 851e.* BLH! photo DAO.)

Elymus smithii (Rydb.) Gould, Madroño 9: 127. 1947.

Agropyron smithii Rydb. var. *typica* Waterf., Rhod. 51: 21. 1949.

Elytrigia smithii (Rydb.) A. Löve, Bot. Not. 1950: 31. 1950.

Perennial, spreading extensively by rhizomes and with a deep fibrous root system. Culms 2.0–9.5 (av. 6.5) dm tall, striate when dry, smooth, glabrous with usually two to five purple nodes. Offshoot leaves in loose tufts, smaller than those of the culm, culm leaves three to five, decreasing in size upwards, bluish green, rarely green or pale green, the sheaths striate when dry, smooth, glabrous, rarely pilose, the blades 2–15 cm long, 1–3 mm wide, rigid and spreading, scabrous on the margin and along the prominent nerves, glabrous, involute when dry. Ligule small, about 0.1 mm long, somewhat erose, glabrous. Auricles 0.2–1.0 mm long, acute, frequently purple. Spikes 6–17 cm long, exserted, the lower four (rarely six) rachis nodes often sterile, the internodes scabrous-margined, the lower two nearly twice the length of the relatively equal subsequent ones, the 6–17 spikelets 14–26 cm long with 2–12 (av. 7) florets, borne singly at a node or occasionally in pairs among any or all of the first six to seven nodes. Glumes one half to two thirds the length of the spikelet, rarely shorter, 9–15 mm long, the first glume slightly longer than the second, acuminate or awn-tipped from below the middle, unequal to oblique, glabrous or variously pubescent, blue-green or partially purpled, the margins membranaceous, three- to five-nerved, the nerves often scabrous. Lemmas, including awn, 9–14 mm long, lanceolate, rounded and smooth or with varying pilosity on the back, acute, mucronate or with an awn 0.5–5.0 (av. 2.0) mm long. Palea slightly shorter than the lemma, the apex erose, the veins finely ciliate. Anthers 2.6–4.4 mm. Caryopsis 4–5 mm long, purple-brown, the tip pilose, adhering to the lemma and palea.

Key to the Forms

A. Lemmas, glumes, and rachis internodes completely glabrous.....f. *smithii*
AA. Lemmas, and/or glumes, and rachis internodes scabrid to pilose.....f. *molle*

AGROPYRON SMITHII Rydb. forma MOLLE (Scribn. & Sm.) stat. nov.

A. spicatum (Pursh) Scribn. & Sm. var. *molle* Scribn. & Sm. in U.S. Dept. Agr. Div. Agrost. Bull. 4: 33. 1897. (Lectotype selected by Hitchcock, Man. Gr. U.S. 1935: *Rydberg 3193* NY, US!)

A. molle (Scribn. & Sm.) Rydb. in Mem. N.Y. Bot. Gard. 1: 65. Feb. 15, 1900.

A. occidentale Scribn. var. *molle* (Scribn. & Sm.) Scribn. in U.S. Dept. Agr. Div. Agrost. Circ. 27: 9. Dec. 4, 1900.

A. smithii Rydb. var. *molle* (Scribn. & Sm.) Jones in Contr. West. Bot. 14: 18. 1912.

Zeia mollis (Scribn. & Sm.) Lunell in Amer. Midl. Nat. 4: 226. 1915.

From the distribution of pubescent specimens as indicated by Fig. 1 and by the behavior of pubescence as described below, the rank of form is adequate for *molle*. To erect further forms based on the occurrence of long awns or such characters as green leaf, purple lemmas, purple anthers, etc. is unnecessary. The pubescent condition is maintained as a form because it has been previously accorded taxonomic recognition.

At the present time an opinion on the status of *A. smithii* var. *palmeri* Scribn. & Smith described from the southwestern United States is deferred until we have more material at hand from that region.

Agropyron smithii Rydb. is readily distinguished from other species of *Agropyron* by means of a few distinctive characters, such as glume shape. There has been confusion, however, with at least two other species, *Agropyron dasystachyum* (Hook.) Scribn. and *Elymus simplex* Scribn. & Williams.

Although the ratio of glume length to lemma length is usually larger in *A. smithii* than in *A. dasystachyum*, that is, the glume of the latter is conspicuously shorter than the first lemma, it is the relative shapes of the glumes that are distinctive. The glumes of *A. smithii* attenuate gradually from below the middle; those of *A. dasystachyum* have parallel sides to above the middle and then abruptly attenuate. Both species have pubescent and glabrous phases affecting chiefly the lemma but sometimes the glume as well, and both are vigorously rhizomatous. While the two characteristically produce little tufts of leaves in the vegetative condition, the attitude of the blades of the flowering culm differ slightly. There is a tendency for those of *A. smithii* to extend outwards at a sharp angle while those of *A. dasystachyum* ascend without any abrupt change of direction. *A. dasystachyum* is tetraploid ($2n=28$); *A. smithii* is octoploid ($2n=56$).

Elymus simplex Scribn. & Williams, considered by Hitchcock (1935) and Hitchcock and Chase (1951) to be a variety of *Elymus triticoides* Buckl., has been confused with *A. smithii*. The typically paired spikelets at the node often considered characteristic of *Elymus* by many authors are lacking in *E. simplex* and the shape of the glume coupled with the orientation of the spikelets to the rachis must be used to distinguish it from *Agropyron*. The

glumes of *E. simplex* taper almost from the base and are slender and usually smooth; those of *A. smithii* taper from below the middle, are wider and prominently ribbed.

The differences between these species are stressed because many herbarium specimens have been found to be misidentified and such an error may account for the report of a $2n=28$ chromosome race of *A. smithii* which we have so far failed to detect.

Geographical Distribution

The map, Fig. 1, gives the distribution of *A. smithii* according to the collections of the Department of Agriculture herbarium in Ottawa supplemented by many records from the New York Botanical Garden and the Gray Herbarium of Harvard University.

The range coincides essentially with that of the Great Plains region with an eastward expansion into the Great Lakes area. Many of the eastern marginal stations coincide with the location of towns and cities and represent local

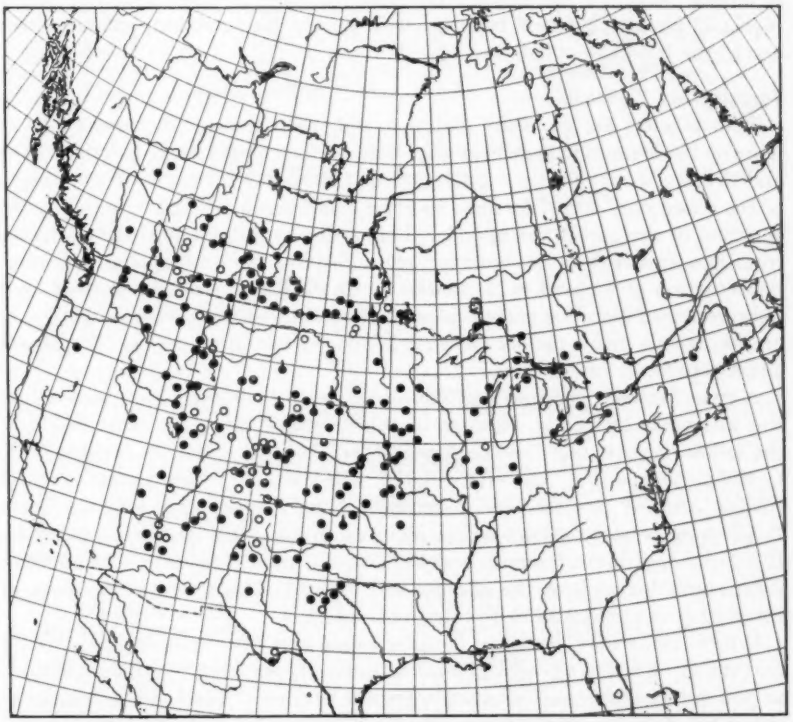

FIG. 1. Distribution of *Agropyron smithii* Rydberg. Solid dots, forma *smithii*. Open circles, forma *molle*. Half dots, both forms occurring together. Tags on dots or circles indicate a chromosome count of $2n = 56$ on material obtained from this location.

introductions rather than natural eastward occurrence of the species, localized either by advance or by recession. The Ontario records are undoubtedly introduction since settlement because the habitat is usually sandy roadsides or railway ballast. At Providence Bay, Manitoulin Island, Ontario, *A. smithii* occurs in loose sand at the upper margin of the beach, along roadsides, and in uncultivated fields. We have no information on the history of the colony but it could have originated from straw shipped with animals from Western Canada and dumped from a lake boat. The colony is fairly extensive and appears to have persisted for several decades.

A collection from west of Port Arthur (*Lindsay 1265*) is considered to be in a patch of relict prairie, but another from 15 miles north of Port Arthur (*Dore & Lindsay 10757*) by cottages on a beach of Lake Superior is considered to be introduced. The stands recorded at Ottawa, viz. Uplands Airport, and nearby Hull, viz. Dupret Park and Wrightville, now extinct through construction work, are considered introductions since the species is absent from likely natural habitats. The Chalk River, Ontario, stand used for study in this paper is almost certainly introduced, probably in feed brought in with horses regularly transported here from the west during the lumbering period at the end of the last century.

Infraspecific Variability

The spikelets are generally glabrous, but when pubescent, the density of hairs on the lemmas is usually greater than on the glumes. Both length of hairs and density may vary or a gradation of pubescence throughout the length of the organ may occur. In some spikes a small patch of hairs is found at the junction of the rachis and spikelet. Population samples deliberately selected from one apparent clone, however, show relative uniformity of pubescence. Examination of a stand of *A. smithii* at Chalk River, Ontario, showed that pubescent and glabrous plants occur in clones but where two dissimilar clones are contiguous then a further clone of slightly pubescent plants may be present either separately or admixed.

A cross between glabrous and pubescent individuals from the Chalk River site was made by W. M. Bowden of this Institute. This was cross B-325 of the following parentage: female parent *1584C2* (*Gillett, Senn, & Bowden 7465*, f. *molle*, bagged but not emasculated, self-sterile); pollen parent *1585C2* (*Gillett, Senn, & Bowden 7466*, f. *smithii*). The F_1 consisted of four seedlings: B-325-A-S1, scabrid (short hairs) lemmas; B-325-A-S2, pubescent lemmas; B-325-A-S3, glabrous lemmas; and B-325-A-S4, scabrid lemmas. The chromosome count for each of the parents was $2n=56$. There was no evidence of correlation of pubescence with other characters.

Lemma awns were measured in order to see how they varied. It was found that the incidence of awned and awnless lemma types follows a pattern similar to pubescence and was consistent in respect to clones. These forms appear in either the pubescent or glabrous phases. Figure 2 indicates the variation in lemma awn length for 12 population samples. In taking these measurements the first lemma was selected from a central spikelet in the spike. The 12

population samples vary widely in representation of awn length classes. Those that are restrictive in class probably represent clonal samples; those that show a wide representation are indicative of wide sampling. The average total percentage histogram indicated a high percentage of individuals with awns

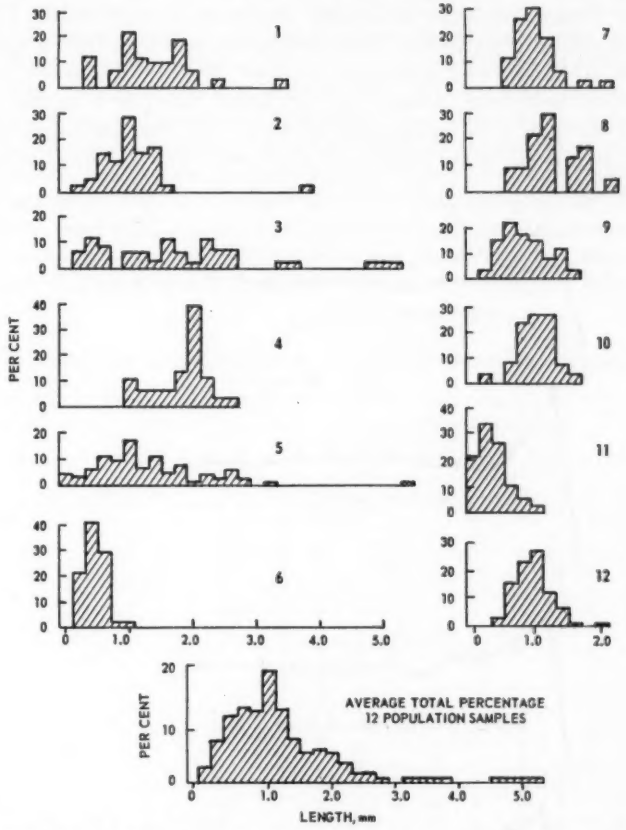

FIG. 2. Variation in lemma awn length as percentage frequency. Each horizontal unit represents a class range of 0.2 mm.

1. *Senn 6215*, Wilsall, Mont. 33 samples
2. *Senn 6193*, Regina, Sask. 42 samples
3. *Senn 6183*, Emerson, Man. 34 samples
4. *Gillett & Bowden 7598*, 29 samples
5. *Gillett & Bowden 7597*, Chalk River, Ont. 67 samples
6. *Gillett & Bowden 7593*, Chalk River, Ont. 61 samples
7. *Senn 6189*, Craven, Sask. 33 samples
8. *Senn 6217*, Sweet Grass Co., Mont. 24 samples
9. *Senn 6257*, Ft. Pierre, S.D. 27 samples
10. *Senn 6246*, Newcastle, S.D. 26 samples
11. *Senn 6261*, Hetland, S.D. 41 samples
12. *Senn 6253*, Rapid City, S.D. 52 samples

of 1 mm or less. In general, this histogram approximated a normal curve and might have approached more closely to it if the sampling method had been confined to individual clones exclusively.

A straight-line relationship might be expected between the number of spike nodes and length of spike. This appears to be the case.

Figure 3 compares spike internodal diagrams of five North American *Agropyron* species populations. These are random selections from a number of diagrams at hand. The similarity between that of *A. smithii* and *A. dasystachyum* is at once evident but the latter differs in a smaller decline following the peak. It is apparent for all species that the first internode from the base of the spike is the longest and that there is a rapid drop to a steady level thereafter.

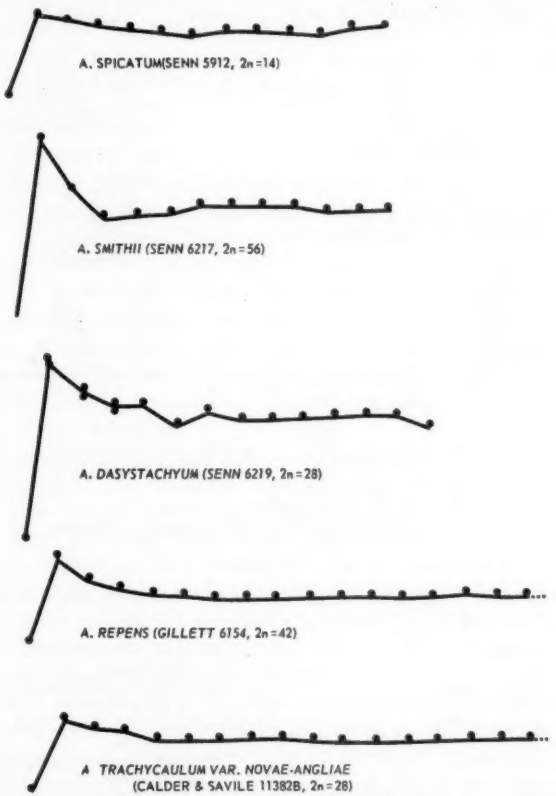
A. SPICATUM(SENN 5912, 2n=14)
A. SMITHII (SENN 6217, 2n=56)
A. DASYSTACHYUM (SENN 6219, 2n=28)
A. REPENS (GILLETT 6154, 2n=42)
A TRACHYCAULUM VAR. NOVAE-ANGLIAE
(CALDER & SAVILE 11382B, 2n=28)

FIG. 3. Comparative spike internodal diagrams. Dots indicate location of spikelets. Horizontal distance—number of nodes; vertical distance—length of internodes.

Spikelet length within the spike shows little variation but spikelets frequently do not develop at the lower nodes of the spike. Of 478 culms from 12 population samples, 57% had no aborted spikelets, 41% had abortion at the first node (numbered from the base of the spike), 18.8% had abortion extending to the second node, 6.5% to the third, 2.9% to the fourth, 0.8% to the fifth, and 0.2% to the sixth. Comparison with *A. dasystachyum* was not possible because there were insufficient population samples of the species. However, similar tabulations with respect to *A. repens* indicate a figure of 80% without sterile nodes, 20% with the first node sterile, 7.5% extending to the second, 2.5% to the third, and less than 2% to subsequent higher nodes, indicating a much lower degree of abortion of lower spikelets in *A. repens* than in *A. smithii*.

Paired spikelets at a node, purported to be a character of the genus *Elymus*, occur. They do not appear to disturb the internodal pattern unduly. A sample of *A. smithii*, *Senn 6189*, consisting of 33 culms, showed 13 with paired spikelets at one or more nodes, numbered from the base of the spike as follows: node 2, 4 culms; node 3, 1 culm; nodes 2 and 3, 3 culms; nodes 3 and 4, 1 culm; nodes

TABLE I

The arrangement of number of florets per spikelet on two population samples of *A. smithii* Rydb.

Sample	Culm No.	Nodes from base											
		1	2	3	4	5	6	7	8	9	10	11	12
Senn 6246, Newcastle, S.D., 22 spikes	1	2	6	6	6	7	7	7	7	8	8	2	—
	2	2	5	5	3	6	8	6	3	3	—	—	—
	3	7	7	7	7	7	4	—	—	—	—	—	—
	4	3	5	5	6	6	6	6	—	—	—	—	—
	5	3	3	7	7	7	8	6	8	8	8	2	7
	6	3	3	7	7	7	8	6	8	8	8	2	—
	7	0	4	5	5	7	6	6	6	7	3	—	—
	8	6	6	7	5	8	7	8	7	5	5	7	—
	9	6	8	10	4	10	—	10	10	—	—	—	—
	10	0	3	5	6	5	6	—	—	—	—	—	—
	11	3	3	6	7	6	—	—	—	—	—	—	—
	12	1	7	7	7	7	8	8	3	7	7	8	—
	13	2	6	3	8	9	8	9	7	8	4	—	—
	15	6	7	9	9	9	11	9	9	9	8	—	—
	16	1	3	4	4	2	2	2	3	—	—	—	—
	17	3	4	4	4	3	5	5	2	6	2	—	—
	18	5	5	6	8	6	7	7	5	2	2	—	—
	19	5	6	7	7	7	7	7	3	—	—	—	—
	20	0	0	1	2	5	2	5	2	2	5	—	—
	22	8	9	9	10	9	2	2	2	9	2	—	—
	23	6	5	6	2	3	6	4	—	—	—	—	—
	24	6	11	11	4	11	4	4	10	2	11	2	7
Gillett 7593, Chalk River, Ontario	1	5	5	6	6	6	7/4*	6	6	6	—	—	—
	2	0	0	3	3	5	4	5	5	5	—	—	—
	3	0	3	3	3	3	4	4	5	4	5	5	—
	4	0	4	4	6	4	5	—	—	—	—	—	—
	5	0	4	5	5	5	5	6	5	2	—	—	—
	6	0	1	3	4	5	4/2*	4	4/3*	5	—	—	—
	7	0	3	4	6	6	5	—	—	—	—	—	—
	8	0	0	5	6	5	5	5	6	5	5	—	—

*Paired spikelets at the node.

2–5, 2 culms; nodes 1 and 3, 1 culm; nodes 2, 4, and 5, 1 culm. A collection by *Calder & Savile 11376* had two of five culms with paired spikelets, one with a paired spikelet at the second node, and one with nodes 1–6 paired. Collection *Senn 6183* showed 8 culms of 34 paired with one each of the following combinations: node 2, node 6, nodes 3 and 5, nodes 2–6, nodes 2 and 3, nodes 2 and 5, node 3, nodes 3–5.

A sample of *A. dasystachyum*, *Senn 6219*, containing 17 culms had three admixtures of *A. smithii*. Of the remaining 14, 6 had no pairing in the spike, 4 had pairs at nodes 2–4, 1 at node 5 only, 1 at nodes 2–6, 1 at nodes 3–5, and 1 at nodes 2 and 3.

Evidently then there is no significant difference in occurrence of paired spikelets in either species. Paired spikelets are apt to occur at one or at several nodes in a variety of combinations. This character is not restricted to *Elymus* and orientation of the spikelet to the rachis is of much greater value to distinguish the genera.

The number of florets per spikelet tends to rise quickly to a constant level from the base to the top of the spike but the increase to maximum number of florets is apt to occur in steps as for example, 4,4,4; 5,5,5; 6,6. This is shown in Table I by reading across the columns. If there is damage to a spikelet at a node caused by ergot or insects, then the number of florets will quickly return to the original level in the subsequent nodes.

Somatic Chromosome Number

Chromosome counts of $2n=56$ for 62 collections of *Agropyron smithii* from widely scattered locations are cited below. The count was the same for both the glabrous and pubescent forms of the species. All determinations have been made by W. M. Bowden unless otherwise indicated.

CANADA. ONTARIO: *1089C1* (*Senn & Lindsay* 5390), *1090C1* (*Senn & Lindsay 5388*) both Providence Bay, Manitoulin Island; *564* determined on the original plant (*Frankton & Forsythe s.n.*, Ottawa); *1506C1* (*Senn 5957*), *1584C7* (*Gillett, Senn, & Bowden 7465*), *1585C1* (*Gillett, Senn, & Bowden 7466*), *1586C1* (glabrous phase *Gillett, Senn, & Bowden 7467*), *1587C1* (pubescent phase *Gillett, Senn, & Bowden 7468*), *1600C1* (*Gillett & Bowden 7596*), *1601C1* (*Gillett & Bowden 7597*) all Chalk River.

MANITOBA: *1616C1* (*Senn 6177*, Woodridge); *1613C1* (*Senn 6183*, Emerson); *208C1* det. M. Heimburger (*Senn 3034*, Turtle Mountain); *212C4* det. M. Heimburger (*Senn & Gordon 3107*, 7 miles east of Medora); *640C1* (*Dore 9216*, St. Jean Baptiste); *643C1* (*Dore 9225*, Domain).

SASKATCHEWAN: *123C1* det. M. Heimburger (*Senn 2320*), *125C2, C4, C5* det. Bowden & Heimburger (*Senn 2322*), *1315S1* (*Senn 5952*), *1316S1* (*Senn & Frankton 5905*) all Maple Creek; *1644C1* (*Senn 6189*, Craven); *1645C1* (*Senn 6192* Valeport); *1646* (*Senn 6195*, Ernfold); *1652* det. on original plant (*Senn 6205*, Govenlock); *141C1* det. M. Heimburger (*Senn 2453*), *143C1, C2, C3, C4*, det. Bowden & Heimburger (*Senn 2455*) both Regina near Wascana Lake; *148C2* det. M. Heimburger (*Senn 2510* Killdeer); *269C3* det. M. Heimburger (*Tisdale 1*), *270C1* det. M. Heimburger (*Tisdale* s.n.) both Swift Current;

285C1 det. M. Heimburger (*Knowles 12*, Humboldt); *292C1* det. M. Heimburger (*Knowles 19*, Hamlin); *297C1* det. M. Heimburger (*Knowles 24*, Unity); *300C1* det. M. Heimburger (*Tisdale s.n.*, Matador).

BRITISH COLUMBIA: *1318S1, S2, S3, S4, S5* (*Senn & Frankton 5876*, Cranbrook); *1638C2* (*Calder & Savile 11376*, Wardner).

UNITED STATES. CALIFORNIA: *1779S1, S2* (*Stebbins 3259*, Shasta Valley, Siskiyou County). COLORADO: *1032S1* (*William & Penland s.n.*, near Colorado Springs). MONTANA: *1653C1* (*Senn 6206*, Havre); *1654C1* (*Senn 6215*, Wilsall); *1656C1* (*Senn 6217*, Livingston). NEBRASKA: *1011S1* (*Newell s.n.*, Lincoln). OKLAHOMA: *1780S1* (received from Division of Plant Exploration, Beltsville, Maryland, PQ No. X36790, Woodward). SOUTH DAKOTA: *1670* det. on original plant (*Senn 6246*, Newcastle); *1672C1* (*Senn 6253*, Rapid City); *1673C1* (*Senn 6254*, Belvidere); *1674* det. on original plant (*Senn 6257*, Ft. Pierre); 1675 det. on original plant (*Senn 6261*, Hetland); *1709S1, S2*, (*Senn 6260*, 28 miles southeast of Wall). TEXAS: *951S1* (from Texas A. & M. College, College Station). WYOMING: *1668* det. on original plant (*Senn 6240*), *1669* det. on original plant (*Senn 6260*) both Spotted Horse. MISCELLANEOUS: *490S1* Seed exchange 47-634-8, from Moscow, U.S.S.R.; *969S1* (Soil Conservation Service, Beltsville, Maryland); *733S1* (B. Rosengurtt, cultivated Montevideo, Uruguay).

Peto (1930) obtained a count of $2n=56$ for *A. smithii* and counts of $2n=56$ and $2n=28$ for *A. smithii molle*. Nielsen and Humphrey (1937) reported a somatic chromosome number of $2n=56$ for *A. smithii* on material grown in the U.S. Soil Conservation Service greenhouses at Ames, Iowa. The source of the material was not given but they stated that they had verified Peto's counts. Hartung (1946) considered that there were two races, a tetraploid and an octoploid. The tetraploid count was determined on material originating at Plush, Oregon, a point which from the distribution map appears to be well beyond the natural range of the species. No voucher specimen was retained and consequently we have not been able to verify the identity of her material. A count of $2n=42$ was recorded by Myers (1947) based on an unpublished report of Stebbins. A living plant of Stebbins' material was obtained and Bowden found the chromosome number to be $2n=56$. Undoubtedly the tetraploid count of Peto was the result of an error in identification as when Senn, Bowden, and Moore (1949) reported the same two counts for this species. In the latter case the tetraploid species proved to be *A. dasystachyum* (Hook.) Scribn. Tateoka (1956) demonstrated the comparative size of chromosomes between species of *Agropyron* and *Brachypodium*. A chromosome count for $2n=56$ was given for *A. smithii* var. *molle*. If tetraploid or hexaploid races exist, they were not detected during our examination of 62 cultures of *A. smithii*.

Meiosis

Meiosis was studied in some collections by R. J. Moore and regular pairing or only minor irregularities were found. Pairing of chromosomes at metaphase I

was regular, 28 bivalents were formed in collections bearing the cytological accession numbers *123C* and *125C, 951S, 1032S, 1506C,* and *1656C.*

Irregularities in pairing consisted of the formation of one quadrivalent, the remaining chromosomes forming bivalents, or the occurrence of two unpaired chromosomes and 27 bivalents. One quadrivalent was regularly formed in collection *208C*; one quadrivalent plus 26 bivalents or 28 bivalents were found in equal frequencies in collection *1584C.* In collection *300C,* 28 bivalents or 27 bivalents plus two univalents were seen at metaphase I. The low frequency and minor nature of pairing irregularities indicate that chromosomal rearrangements are probably slight throughout the species. Regular meiotic pairing is illustrated in Fig. 4A (collection *564C*) and, in Fig. 4B, the formation of 26 bivalents plus one quadrivalent (collection *208C*).

Moore attempted to make the following crosses: *A. smithii* × *A. dasystachyum* employing *125C* × *127C6, 123#3* × *127#3, 123#4* × *205C, 125C1* × *165#1, 125#5* × *162#3,* and *125C1* × *162C1*; the reciprocal *165C1* × *212C4*; two crosses of *A. smithii* × *A. trachycaulum, 208* × *192S14,* and *125C6* × *149C3*; four of *A. smithii* × *A. intermedium, 125C1* × *94S1, 125#5* × *94S1, 125#3* × *94S2, 212C2* × *94S*?; the reciprocal *94S1* × *300C*; three of *A. smithii* × *A. cristatum, 208* × *67S3, 125#4* × *142S2, 125#6* × *67S3*; and one cross of *A. smithii* with *A. repens, 125C6* × *88.* No hybrid seed was obtained as a result of these attempts.

Two infraspecific attempts by Moore to cross *A. smithii* employing *148#2* × *125#2* and *208* × *125C6* produced no seed. Bowden successfully crossed f. *molle* and f. *smithii* as reported earlier in this paper and the cross B-229-S1 of the following parentage: *1089* (*Senn & Lindsay 5390,* f. *smithii,* not emasculated, self-sterile) × *123C4* (*Senn 2320,* f. *smithii*) was successful also and produced one seed. Originally this cross was designed to test the effect of crossing purple and yellow anther forms but the female parent with purple anthers produced yellow anthers when grown in the greenhouse.

No seed was produced when Bowden selfed the following material: Cyt. No. *212C1* (*Senn & Gordon 3107,* Medora, Man.), *1315S5* (*Senn 5952,* Maple Creek, Sask.), *1316S4* (*Senn & Frankton 5876,* Cranbrook, B.C.), *1506* (*Senn 5957,* Chalk River, Ont.), *1584C1, 1584C9* (*Gillett, Senn, & Bowden 7465,* Chalk River), *1585C1, 1585C4* (*Gillett, Senn, & Bowden 7466,* Chalk River). However *1089* (*Senn & Lindsay 5390,* Manitoulin Island, Ont.) was self-sterile but set a fair amount of seed when wind-pollinated. These results accord with those of Smith (1944), who found that *A. smithii* produced very few seeds when subjected to self-pollination.

These negative results lend support to the belief that effective sterility barriers exist between *A. smithii* and other species tested. If self-sterility is also present then the species must persist chiefly by vegetative means and by seeds produced by cross-pollination.

Lepage (1952) described ×*Agroelymus turneri* as a hybrid with the putative parentage *Agropyron smithii* ×*Elymus innovatus.* This hybrid was considered a synonym of ×*Agroelymus hirtiflorus* (Hitch., pro sp.) Bowden by Bowden

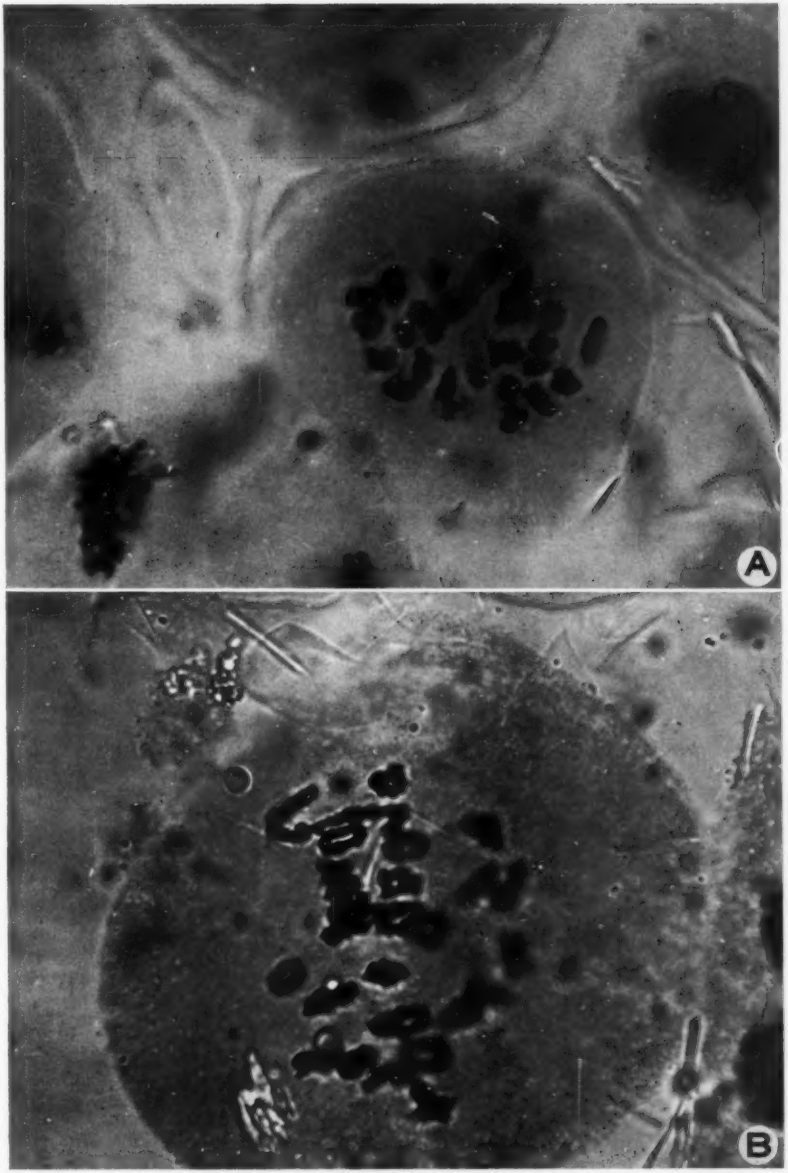

FIG. 4. Pairing at metaphase I in *A. smithii* Rydb. A. Regular (collection 564C). B. Irregular (collection 208C).

(1959). Because *A. smithii* has proved to be consistently octoploid and because no crosses employing *A. smithii* have been successful, then *A. smithii* was not a parent of this hybrid. As Bowden (1959) stated, the *Agropyron* parent was either *A. trachycaulum* (Link) Malte or *A. dasystachyum* (Hook.) Scribn. in one of their several forms.

Discussion

Agropyron smithii is the only octoploid species of *Agropyron* in North America. Evidently *A. smithii* has reached the peak of the polyploid series in North America and with its effective sterility barriers is not likely to evolve further by hybridization. Since it is native on this continent it likely arose here, and probably in one of two ways, either by doubling of a tetraploid species or by crossing of two tetraploids with subsequent doubling to form an allopolyploid species. From general morphology such as the presence of rhizomes, leaf type, similarity of spike pattern, and behavior of pubescence, *A. dasystachyum* may be a possible progenitor. One of the most interesting and distinctive morphological characters of *A. smithii* is the shape of its glume, a shape which is similar to the glumes of species of *Elymus*. A species of *Elymus* then should be considered as the other parent.

By and large, *A. smithii* is a stable species. Extremes of color, pubescence, and awning appear to occur within the normal range of variation expected. Because of the high level of polyploidy, many recombinations of these characters are to be expected but no one character or combination of them appears to be confined to a geographical region. Long-awned or pubescent forms may occupy local areas by the production of vegetative clones.

The pattern of variation shown by *A. smithii* may be expected in other species of *Agropyron* and should be considered in the study of them.

Acknowledgments

The authors wish to acknowledge the work of the several cytologists who have made chromosome counts cited in this paper. The bulk of them were made by Dr. W. M. Bowden. Earlier counts were the work of Dr. Margaret Heimburger (née M. Landes); Mrs. Shu-Chang Lin made a few counts at a later date. Crossing and meiosis data are by Dr. R. J. Moore; selfing data are by Bowden. All persons are or have been members of the staff of this Institute or the former Division of Botany and Plant Pathology.

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AN INDOLEACETIC ACID 'OXIDASE' SYSTEM IN THE MYCELIUM OF MELAMPSORA LINI (PERS.) LEV.[1]

ANN OAKS AND MICHAEL SHAW

Abstract

Flax rust mycelium was grown on cotyledons of flax in a modified Knop's medium. The mycelium produced and apparently secreted a typical peroxidase-mediated indoleacetic acid 'oxidase'. Decarboxylation of radioactive indoleacetate (—$C^{14}OOH$) was stimulated by Mn^{++} (10^{-4} and 10^{-3} *M*), dichlorophenol (10^{-4} *M*), and resorcinol (10^{-4} *M*). Catechol (10^{-4} *M*) and pyrogallol (10^{-4} *M*) inhibited the reaction. Moreover, pyrogallol competitively inhibited the reaction in the presence of resorcinol. All four phenols increased oxygen uptake by the mycelium, catechol and pyrogallol being the most effective. Cu^{++} (10^{-3} *M*) inhibited the reaction by 60%; cyanide (10^{-3} *M*) and diethydithiocarbamate (2×10^{-4} *M*) were almost completely inhibitory. The results are discussed briefly in relation to host–parasite interactions and the control of indoleacetic acid oxidase activity in vivo.

Introduction

The indoleacetic acid oxidase system has been considered to be an important factor in controlling the level of endogenous indoleacetic acid in healthy (6, 14) and rust-infected (12, 13, 19) tissue. More information concerning the action of the oxidase system in intact leaf tissue and in the mycelium of the rust fungi is therefore much to be desired.

The aerial mycelium produced by flax rust on the cotyledons of flax in nutrient medium (25) was found to have an indoleacetic acid oxidase system which can be studied 'in vivo'. The opportunity was therefore taken to carry out the following experiments, which are of interest not only in connection with indoleacetic acid metabolism per se, but also in connection with the host–parasite relations of the rust fungi (12, 19).

Methods

The ectomycelium of *Melampsora lini* (Pers.) Lev. was grown on the cotyledons from flax seedlings (varieties Bison and Redwing) in a modified Knop's medium and was supplied by Dr. F. L. M. Turel. The mycelial mat was easily separated from the host tissue with forceps and was used as the experimental material.

The decarboxylation of radioactive indoleacetic acid (—$C^{14}OOH$, supplied as the potassium salt (see ref. 18)) and oxygen uptake were determined with a Warburg apparatus at $25\pm0.02°$ C in darkness. Three hours before the start of each experiment approximately 5 milligrams of fresh mycelium were placed in each Warburg flask with 2.1 ml of 0.05 *M* phosphate buffer at pH 5.2. At the end of this preincubation, manganese (10^{-3} or 10^{-4} *M*) and either dichlorophenol (10^{-4} *M*) or resorcinol (10^{-3} or 10^{-4} *M*) were added and

[1]Manuscript received March 28, 1960.
Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask.

the flasks were shaken at 100 oscillations per minute through a 4-cm path for 30 minutes in the water bath. Measurements of oxygen uptake were corrected for the autoxidation of phenols in the presence of mycelium that had been inactivated by heating at 80° C for 5 minutes.

Radioactive indoleacetate (30 μg) was added from the side arm to give a fluid volume of 3.0 ml in the reaction chamber of each flask. Radioactive carbon dioxide liberated by the oxidative decarboxylation of the indoleacetate was trapped in potassium hydroxide (0.2 ml 10% solution) in the center wells, collected at the end of the incubation period, and converted to barium carbonate for measurements of radioactivity. All measurements of activity were made to a standard error of 2%.

Preliminary tests showed that the rate of destruction of the indoleacetate was approximately linear for the first 3 hours. Incubation was normally continued for only 2 hours, and the measurements of decarboxylation during this period (counts per minute per 5 mg mycelium) are here regarded as 'initial' rates of 'oxidase' activity. (See Tables I and III.)

Results

Indoleacetic acid was oxidized by flax rust mycelium (Table I). The oxidation was stimulated by the addition of manganese (10^{-4} *M*) and dichlorophenol (10^{-4} *M*) and was prevented by heating at 80° C for 5 minutes. When the mycelium was placed on sterile agar (1%) overnight an enzyme system with similar properties was recovered from the agar. Thus, a system capable of oxidizing indoleacetic acid was released from the mycelium under these conditions, though it has not been shown that such 'secretion' occurs in the complete absence of wounding.

In the presence of manganese (10^{-4} *M*), both dichlorophenol and resorcinol enhanced the oxidation of indoleacetic acid by the mycelium of flax rust (Table I). Catechol and pyrogallol inhibited the oxidation (Table I). All four phenols increased the rate of oxygen uptake, but catechol and pyrogallol were the most effective (Table I). In the presence of pyrogallol or catechol both the medium and the mycelium turned brown in color during the experimental period of 2–3 hours. Ascorbic acid (10^{-2} *M*), which was itself oxidized by the mycelium, depressed the rate of browning.

TABLE I

The effect of phenols on the oxidation of indoleacetic acid and on oxygen uptake by flax rust mycelium

Treatment	O_2 uptake* (μl per 5 mg per hour	IAA 'oxidase' (c.p.m. per 5 mg per 2 hours)
Control	0.5 (approx.)†	735
+ dichlorophenol (10^{-4} *M*)	5	5613
+ resorcinol (10^{-4} *M*)	6	4637
+ catechol (10^{-4} *M*)	8	106
+ pyrogallol (10^{-4} *M*)	17	28

*Corrected for autoxidation of phenols.
†Assuming a dry weight of 10%, this corresponds to 10 μl per 10 mg dry wt. per hour.

In the presence of resorcinol, manganese increased the rate of oxygen uptake and the oxidation of indoleacetic acid (Table II). Copper had no effect on oxygen uptake, but inhibited the destruction of auxin (Table II). On the other hand, the oxidation of pyrogallol was slightly stimulated by copper, but was inhibited by manganese (Table II).

TABLE II

The effect of Cu and Mn on the oxidation of pyrogallol and indoleacetic acid

Treatment	Pyrogallol 'oxidase'*		Resorcinol – IAA 'oxidase'†			
	O_2 uptake	%	O_2 uptake	%	c.p.m.	%
Control	15	100	4	100	1020	100
Mn (10^{-3} *M*)	9	60	12	300	8865	869
Cu (10^{-3} *M*)	19	120	4	100	409	40

NOTE: Corrected for autoxidation of phenols. Oxygen uptake = µl O_2 per 5 mg fresh wt. per hour.
*Pyrogallol concentration, 10^{-3} *M*.
†Resorcinol 10^{-3} *M* plus 30 µg radioactive indoleacetate.

Cyanide and diethyldithiocarbamate (dieca) almost completely inhibited the oxidation of indoleacetic acid (Table III). Thus the oxidation of indoleacetic acid by flax rust mycelium is mediated by a heavy metal enzyme. Reinert *et al.* (17) showed that dieca removes manganese from an indoleacetic acid oxidase system in extracts from pea epicotyls. This may have occurred here also. Since the oxidation is stimulated by manganese and monophenols, and inhibited by copper and polyphenols, peroxidase is probably the enzyme involved (9, 22, 26).

The oxidation of pyrogallol was strongly inhibited by cyanide (63%) and mildly inhibited (32%) by dieca (Table III). These results suggest the action of both oxidases and peroxidase. The oxidation of pyrogallol is, however, stimulated by copper, which may be necessary as a cofactor. Dieca may act by removing this, rather than by reacting with the copper component of an enzyme.

The foregoing results show that copper and manganese had opposite effects on the oxidation of indoleacetic acid and pyrogallol (Table II). Moreover, pyrogallol depressed the rate of oxidation of indoleacetic acid but increased oxygen uptake (Table I). It thus appeared that the oxidation of pyrogallol might competitively inhibit the oxidation of indoleacetic acid.

TABLE III

The effect of cyanide and diethyldithiocarbamate on the oxidation of pyrogallol and indoleacetic acid

Treatment	Pyrogallol 'oxidase'*		IAA 'oxidase'†	
	µl O_2 per 5 mg per hour	%	c.p.m.	%
Control	22	100	1972	100
+ dieca (2×10^{-4} *M*)	15	68	162	6
+ KCN (10^{-3} *M*)	8	37	277	12

NOTE: Oxygen uptake corrected for autoxidation of phenols.
*Pyrogallol concentration, 10^{-3} *M*.
†10^{-3} *M* resorcinol plus 30 µg radioactive indoleacetate.

The oxidation of a range of concentrations of indoleacetic acid was therefore measured in the presence and absence of pyrogallol (10^{-5} *M*). Assuming Michaelis kinetics the reciprocal of the rate of decarboxylation was plotted against the reciprocal of the concentration of indoleacetic acid (11). Since the slope of the line is very much steeper in the presence than in the absence of pyrogallol (Fig. 1), the system oxidizing indoleacetic acid appears to be competitively inhibited. Rabin and Klein (15) have obtained results which suggest that chlorogenic acid, also a polyphenol, competitively inhibits the oxidation of indoleacetic acid by extracts obtained from pea epicotyls. Conversely, Siegal found that the oxidation of pyrogallol by horse-radish peroxidase (20) and by non-enzymatic (21) systems was depressed by indoleacetic acid. These results would be expected if the action of pyrogallol on the oxidation of indoleacetic acid were competitive.

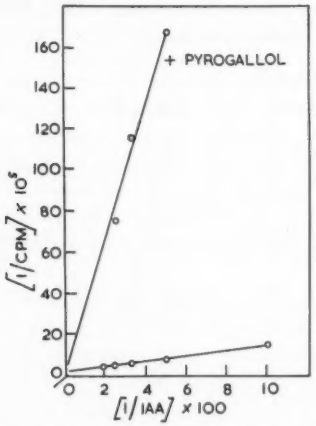

FIG. 1. The effect of pyrogallol on the oxidation of indoleacetic acid. Ordinate: Reciprocal of IAA oxidase × 10^5. Abscissa: Reciprocal of IAA concentration × 10^3. The mycelium was placed in 0.05 *M* phosphate buffer (pH 5.2) 3 hours before the addition of substrate. Each flask contained manganese (10^{-4} *M*) and resorcinol (10^{-4} *M*). Pyrogallol (10^{-5} *M*) and 30 μg of radioactive indoleacetate were added after equilibration. The results are calculated from mean values for duplicate experiments.

Discussion

Two fungi, *Polyperus versicolor* (4, 24) and *Omphalia flavida* (16, 18), have previously been shown to produce and secrete an indoleacetic acid oxidase. Moreover, secretion of the enzyme is clearly responsible for the defoliation of coffee by *Omphalia* (18).

The data in Tables I, II, and III strongly suggest that the indoleacetic acid oxidase produced and apparently secreted by *Melampsora lini* is a typical, peroxidase-mediated system (9, 22, 26). Germinating uredospores of *Puccinia graminis* produce a similar system (12). Thus, production of an indoleacetic acid oxidase by the fungus may account for the enhanced destruction of indoleacetic acid by wheat leaf disks observed in the early stages of infection

with *Puccinia graminis* (19) and by dialyzed extracts of leaves at all stages of infection (12). Since infection with obligate parasites usually raises the level of extractable auxin (3, 13, 19), it appears that the activity of the oxidase is controlled by factors developed by either the host or parasite or both. An accelerated synthesis of auxin may also be a contributory factor. Pilet (13) found that although extracts from aecial conceptacles of *Uromyces pisi* on *Euphorbia cyparissias* exhibited a relatively constant high activity of indoleacetic acid oxidase, the activity in extracts from infected tissue fell as the intensity of infection rose. Moreover, the addition of crude aecial extracts to crude extracts of healthy leaves invariably lowered the oxidase activity of the latter. Thus Pilet considered that the increased auxin content of infected leaves of *Euphorbia* was the result of inhibition of oxidase activity by non-specific 'toxins' elaborated by the parasite.

Indoleacetic acid, many phenols, and ascorbic acid are all oxidized by systems that are mediated by either peroxidase (9, 22, 26) or copper oxidases (2, 23). The concentrations of phenolic or other cofactors or inhibitors are often more important in controlling the rate of indoleacetic acid oxidation than is the activity of peroxidase (8). The decarboxylation of indoleacetic acid by leaf disks of a susceptible variety of wheat was depressed after the development of rust infections (19). In contrast, destruction of the auxin by crude leaf extracts as well as by dialyzed extracts, containing manganese and dichlorophenol, was much higher after rust infection (12). It is apparent that a factor other than the enzyme concentration must be controlling the over-all activity of the indoleacetic acid oxidase system in vivo. It is also apparent that if the activity in intact rusted tissue is low because of the presence of inhibitors or the relative lack of cofactors, then the relative concentrations of inhibitors and cofactors must be altered by the process of extraction so as to result in an enhanced activity.

The apparent effect of pyrogallol in inhibiting the oxidation of indoleacetic acid by *Melampsora* (Fig. 1, Table I) illustrates another mechanism that may operate to control the auxin level. The oxidation of both pyrogallol and indoleacetic acid appears to be mediated by peroxidase. Thus, competition between several oxidase systems having one or more steps in common may be envisaged. The outcome of such competition may be expected to depend upon the relative affinities of the enzyme for its immediate substrates and on the relative concentrations and degree of oxidation of the various redox pairs participating in each system. Phenolic substances and metallic cations that alter the ratio of oxidized to reduced forms of any redox pair in the system, or that participate in it, may thus control the rate of electron transfer along the various carriers.

The interaction between the pyrogallol and indoleacetic acid oxidase systems may represent either a competition between mono- and poly-phenols for active sites on the enzyme, as described by Gortner and Kent (7) for ferulic and *p*-coumaric acids or, less specifically, the ease with which the electrons are released by the various catalysts. A comparison of the rates of

enzymatic (10) and non-enzymatic production of manganipyrophosphate (1) indicates the importance of the relative rates of availability of electrons in determining the outcome of competing reactions. In both these cases monophenols stimulate the reaction and the more easily oxidized polyphenols inhibit it.

If the in vivo balance between competing oxidase systems does in fact depend in part on the relative concentrations of activators and inhibitors and in part on the ratio of oxidized to reduced forms of particular electron carriers, it would not be surprising if this balance were markedly altered during the preparation of extracts. This could easily explain the lack of correlation often found between indoleacetic acid oxidase activity in intact tissue and in extracts from it.

The work of Flaig and Saalbach (5) provides indirect evidence that phenolic compounds may establish certain oxidation reactions in whole tissues. They found that hydroquinone, a well-known inhibitor of the peroxidase – indoleacetic acid oxidase system in plant extracts (15) stimulated growth, respiration, and the activity of enzymes oxidizing ascorbic acid in cress roots. These are conditions that promote the oxidation of ascorbic acid and conserve indoleacetic acid. The possible importance of competing oxidase systems in preserving indoleacetic acid in intact tissue is thus indicated. Such processes may play a role in determining the success or failure of an obligate parasite in establishing itself upon its host.

Acknowledgments

Grants from the National Research Council and the Canada Department of Agriculture to one of us (M.S.) are gratefully acknowledged.

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THE VEGETATION OF NORTHERN MANITOBA

VI. THE LOWER HAYES RIVER REGION[1]

J. C. RITCHIE

Abstract

The vegetation of the Hudson Bay Lowlands of Canada is known only in the most superficial terms. This contribution provides descriptions of the chief cover types of a small portion of the Lowlands near the confluence of the Hayes and Nelson rivers. The area has a continental, subarctic climate. Postglacial marine inundation has deposited thick calcareous clays; the topography is flat.

On the mineral substrata of alluvial and marine deposits the chief types of vegetation are salt marsh, shrub (dominated by *Salix* species), forests of *Populus balsamifera*, and forests of *Picea glauca*. They often form discrete zones in the order mentioned, and it is likely that this is a seral as well as spatial relationship. On the extensive peat substrata there are bog and fen types. In the former are distinguished two communities dominated by *Picea mariana*—one, termed lichen muskeg, with a ground layer of *Cladonia* and *Ledum*, and the other, moss muskeg, with *Pleurozium* and *Ledum groenlandicum* as ground layer dominants. Locally, areas of lichen muskeg have been destroyed by fire and are now occupied by willow communities. In wet inland areas, moss muskeg and lichen muskeg communities are associated with palsa and string-bog features. On shallower peats, usually nearer the shores and rivers, fens predominate, with *Larix laricina* and *Betula glandulosa* as the dominant phanerophytes.

All these types have been distinguished on vertical aerial photographs, and their photographic characteristics are summarized. A detailed vegetation map illustrates their chorology.

Introduction

In a general account of the vegetation of northern Manitoba (18) the area occupied by the Hudson Bay Lowlands is considered, at least tentatively, as a separate vegetation region, and some indication is given of the complexity of the plant communities encountered there. In Manitoba this region is the poorest known botanically, and this is probably true for the Lowlands as a whole compared to other regions of Canada. However, knowledge of flora is far in advance of that of vegetation, for while several scientists and naturalists have visited the region to collect and record both flora and fauna, very few systematic studies of vegetation have been attempted. In Manitoba, contributions by Johnson (13), Cheney and Beckel (6), Ritchie (17), and Schofield (20) have thrown some light on the nature of the vegetation of the Lowlands in the vicinity of Churchill. In Ontario, Hustich (12), Sjörs (24), and Moir (14) have made vegetation studies of varied intensity and detail in the Lowlands region.

The vegetation of subarctic Canada is known only in superficial aspects, but the Hudson Bay Lowlands have intrinsic features which, together with the general scarcity of geobotanical investigations in boreal and subarctic Canada, produce a remarkable lacuna in knowledge. As Coombs (7), who has provided the only systematic account of the geography of the region, and

[1]Manuscript received February 25, 1960.
Contribution from the Department of Botany, University of Manitoba, Winnipeg, Manitoba.

Sjörs (24) point out, it is the physiographic nature of the Lowlands which has restricted study. Navigable channels (by canoe) are few and the interfluves are covered by flat expanses of wet bogs and fens.

This account reports on the vegetation of a small area surrounding the lower stretches of the Hayes River (Fig. 1). The area was selected, firstly because an inspection of the vertical aerial photographs of the entire Lowlands in Manitoba revealed that several representative cover types could be found there, and secondly because it was known that reasonable access to the interior by canoe could be expected.

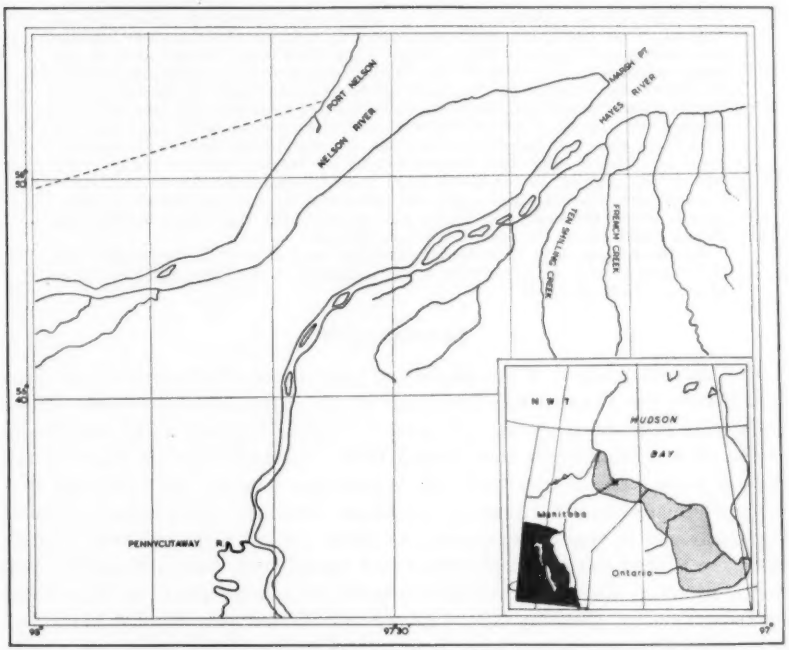


FIG. 1. A map of the area studied, showing the main localities mentioned in the text. The dotted line indicates the position of the abandoned railway right of way. The inset map shows the position of the study area (small black rectangle), the Hudson Bay Lowlands (stippled), the Canadian Shield (unshaded), and the Interior Plains (shaded).

Before the fieldwork was started some useful preliminary notions about the general nature of the country and flora were culled from the reports of Bell (3, 4), Preble (15), Rae (16), and Scoggan (21), and a set of vertical aerial photographs (scale 1:60,000) covering the area was procured and studied. Preliminary mapping of cover types was begun with these photographs, and they made easier the task of selecting particular localities for field study. The photographs were used in the field and proved to be of immense value in this difficult terrain.

One of the first objectives was to establish what types of vegetation predominate, where they are found, and to record this information in the form of a vegetation map. Accordingly, areas on the photographs of distinct tone and texture were outlined in pencil and used as working categories. As many examples as possible of each category were visited and studied, enabling a general, qualitative assessment to be made of their degrees of uniformity. A base map at the scale of the photographs was provided by enlarging with pantograph the appropriate area on the Topographical Map Sheet (scale 1:506,880). The centers of the photographs were located on the base map and the outlines of the vegetation units traced off a mosaic of the photographs. Inaccuracies in the map are due to the excessive enlarging with pantograph, and the corrections necessary in tracing from photographs to base map. It transpired that most of the communities recognizable and mappable at the scale of the photographs were sufficiently homogeneous to sample by the methods used. The term homogeneous is used in the loose, imprecise way, based on personal judgement, and indicating that a particular stand gives the appearance of floristic and structural uniformity which would lead one to consider it as one major plant community.

Sampling was done by selecting an area, 20 m × 20 m, and making a complete list of the vascular plants, mosses, and lichens found, estimating for each layer the cover and sociability of the species, according to the following familiar scales.

Cover (% of the ground covered)	Sociability
+ = up to 1%	1 = growing one in a place, singly
1 = 1 to 9%	2 = grouped or tufted
2 = 10 to 24%	3 = in troops, small patches, colonies
3 = 25 to 50%	4 = in small colonies, extensive patches, or forming carpets
4 = 51 to 75%	
5 = 76 to 100%	5 = in great crowds, pure populations

The heights, ages, and diameters at breast height (the last abbreviated as d.b.h. throughout this account) were recorded, as well as general observations on the substratum, including determinations of reaction with a portable pH meter with combination glass electrodes. Specimens of all species were collected, pressed, and dried, in addition to any plants which could not be identified in the field.

The results of this primary survey are presented below, in the form of a vegetation map (Fig. 2), tabular summaries of community analyses, photographs, and brief verbal descriptions of each type.

I am greatly indebted to Mr. Gordon M. Johnston for excellent collaboration in the fieldwork, and to Drs. Howard A. Crum, Lewis E. Anderson, and John W. Thomson for assistance with identification of non-vascular cryptogams. This study is being supported by grants from the National Research Council of Canada and the Faculty of Graduate Studies and Research of the University of Manitoba.

Physiography and Climate

The area is underlain by horizontally bedded Palaeozoic limestone (1), and the boundary between this and the Pre-Cambrian rocks to the west is usually taken as the limit of the continental section of the Lowlands (8). The vicinity of the lower Hayes and Nelson rivers shows little relief, sloping gently to the sea with a drop of about 0.5 m in 1 km (2). The only relief is provided by raised beaches. Overlying the bedrock, which never outcrops here, there is a thick mantle of marine clay and drift. The steep banks of the trench formed by the Hayes River show a layer, 7 to 9 m in thickness, of stratified, blue-gray clay over a layer of dark-brown boulder clay (glacial).

Coombs (7) includes all of the Manitoba part of the Lowlands, except for a narrow coastal strip, in his Marine Clay Zone, which is so named "because of the widespread mantle of clay deposited during the postglacial marine submergence". As Figs. 3 and 4 illustrate, the drainage is immature, with large tracts of very wet terrain between the few main watercourses.

In the absence of records from the actual precincts, the most useful comparative information on the climate of the area can be found in the climatic maps of the Atlas of Canada (1). The following table of approximate values has been abstracted from this source by extrapolation.

In the Thornthwaite system of classification of climate, the area falls in the humid, microthermal, subarctic region (1), characterized by cool, short summers. From the map of Saunderson (19) it appears that the potential evapotranspiration for the area (defined in ref. 19 as "the amount of water that would be transferred from the soil to the atmosphere by evaporation and transpiration if it were constantly available in optimum quantity") is approximately 32 cm, a value typical of the subarctic region as a whole.

TABLE I

Certain climatic data for the Lower Hayes River Region

Mean daily temperature	January	−25° C
	April	− 8° C
	July	13° C
	October	0° C
*Mean annual number of degree days above 5.5° C		1200
Mean annual frost-free period		70 days
Mean annual total precipitation		32 cm
Mean annual precipitation as snow		200 cm
Mean growing season precipitation		18 cm

*The sum of the daily values during the year of the differences between the daily mean temperature and 5.5° C when the former exceeds 5.5° C.

Vegetation

The main findings are embodied in the vegetation map (Fig. 2), and the clearest picture of the vegetation chorology of the area can be gained by an examination of the map. Ten vegetation categories are mapped. All can be recognized on the photographs, and a summary of their photographic characteristics is provided in Table II. These categories, with one addition, have

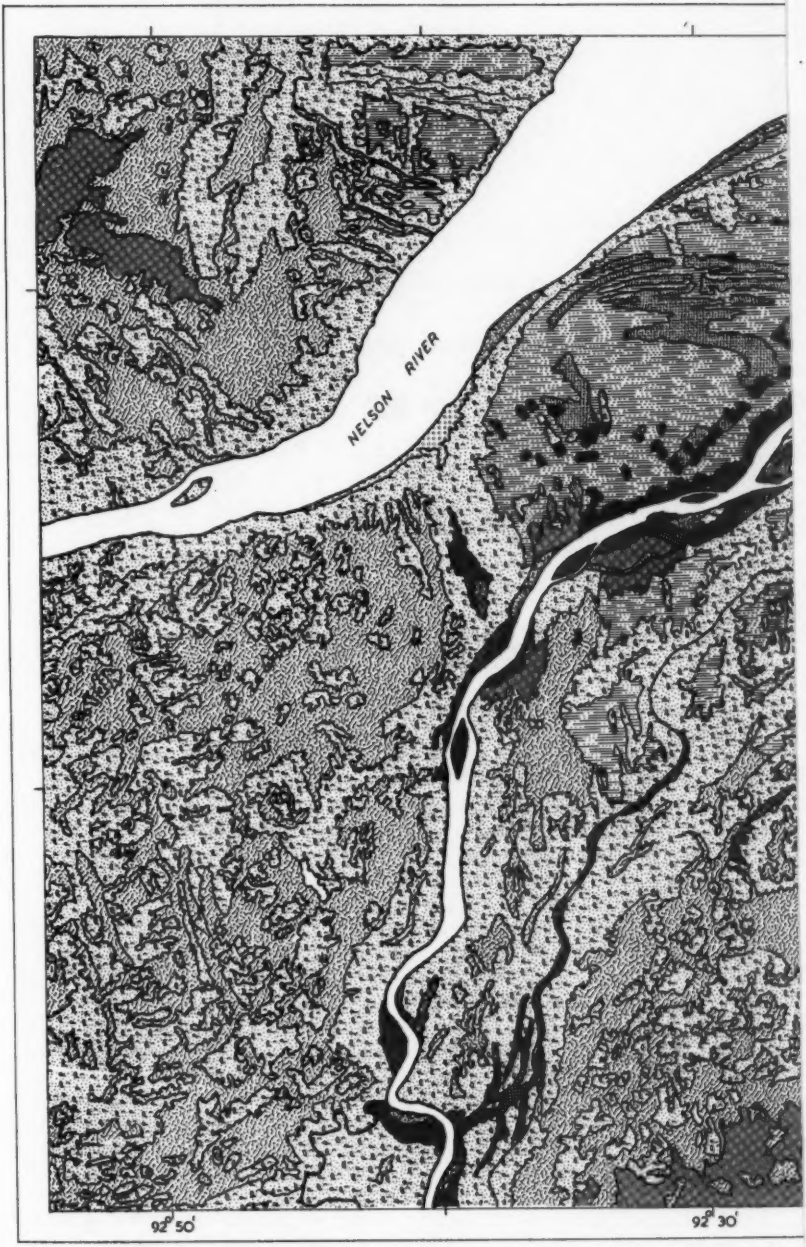


FIG. 2. A vegetation map of the area of study. Details of compilation are given in the t
spruce forest at the narrowest part of the headland between the Hayes and Nelson rivers
confluence of the rivers. Scale 1.250,000.

the area of study. Details of compilation are given in the
part of the headland between the Hayes and Nelson river
.250,000.

- WHITE SPRUCE FOREST
- POPLAR FOREST
- SHRUB ON ALLUVIUM
- MARSH
- SHRUB ON PEAT
- LICHEN MUSKEG
- MOSS MUSKEG
- PALSA AND STRING BOG
- LARCH FEN
- SHRUB FEN

ion are given in the text. It might be noted here that the patch of white
es and Nelson rivers provides interesting botanical evidence of a former

TABLE II

A summary of the main features of the vegetation types as seen on vertical aerial photographs

Vegetation type	Tone	Texture	Photographic appearance	Other features
1. White spruce forest	Black to very dark gray (N2-3)	Medium to coarse stippling	Individual spire-shaped trees usually visible. Individual tree shadows visible	Here confined to recent alluvium, particularly river islands
2. Balsam poplar forest	Very dark gray to dark gray (N3-4)	Almost smooth surface, slightly undulating locally	Individual crowns not visible. Forming discrete patches with even, rounded, or crenate margins. Marginal stand shadow visible	Same sites as 1
3. Shrub stands	Dark gray (N4)	Even surface	Flat surface, broken only by drainage features. No individual shrubs visible: no shadows	On recent alluvium, beaches, wet fens, and after fire on deeper, drier peats
4. Marsh	Light gray (N7)	Even surface with very coarse texture only where cover is broken, as by pans and drainage channels	Discontinuous cover, with small patches of darker tones (water). Coastal margin coarsely fringed (erosion effects)	On the youngest coastal flats
5. Lichen muskeg	Light gray to white (N7 to 8)	Irregular surface with coarse stippling of spaced trees	Well-spaced small spruces visible against white background which is continuous except for small depressions where local bog or shrub vegetation occurs as darker patches	Occurs as (*a*) large island-like patches with long axes up to and exceeding 1 km, and (*b*) continuous belts along river and stream banks and older beaches
6. Moss muskeg	Gray (N6)	Irregular with a less coarse stippling than 5	Partly open to closed stands of stunted trees, similar to 5 but with darker shrub background and more dense stands	Occupying positions similar to 5
7. Palsa and string bog	Light gray on gray (N7 to 8 on N6)	Coarsely pitted surface	Small island-like mounds, sometimes slightly orientated in chains with the depressions occupied by water or wet bog. Occasionally forming long ridges (string-bog) running parallel and at right angles to drainage	Forming large areas in the wettest parts of the oldest, deposits usually with 5
8. Larch fen	Gray (N5 to 6)	Smooth, diffuse broken only by changes in drainage	Large, uniform areas, with individual trees not visible. Surface broken only by ponds and channels	Generally on younger sites near the coasts or rivers. Rarely a pattern of ridges is seen

been grouped below into two types, depending on the general nature of the substratum. In this simple scheme, substrata are distinguished primarily as those with more than 40 cm of peat (*b*) and those with less (*a*), following the generally accepted, at least in northwest Europe (cf. Sjörs (23)), definition of peatland by Granlund (9).

(*a*) Vegetation types developed on predominantly mineral substrata
1. *Picea glauca** forests
2. *Populus balsamifera* forests
3. Shrub communities
4. Marshes
5. Minor communities (fragmentary, not mapped in Fig. 2)

(*b*) Vegetation types developed on predominantly peaty substrata
1. Lichen muskeg
2. Moss muskeg
3. Secondary communities
4. Palsa and string bog

} Bog types

5. Larch fen
6. Shrub fen

} Fen types

*Vascular plant nomenclature accords throughout this paper with that of Scoggan (22).

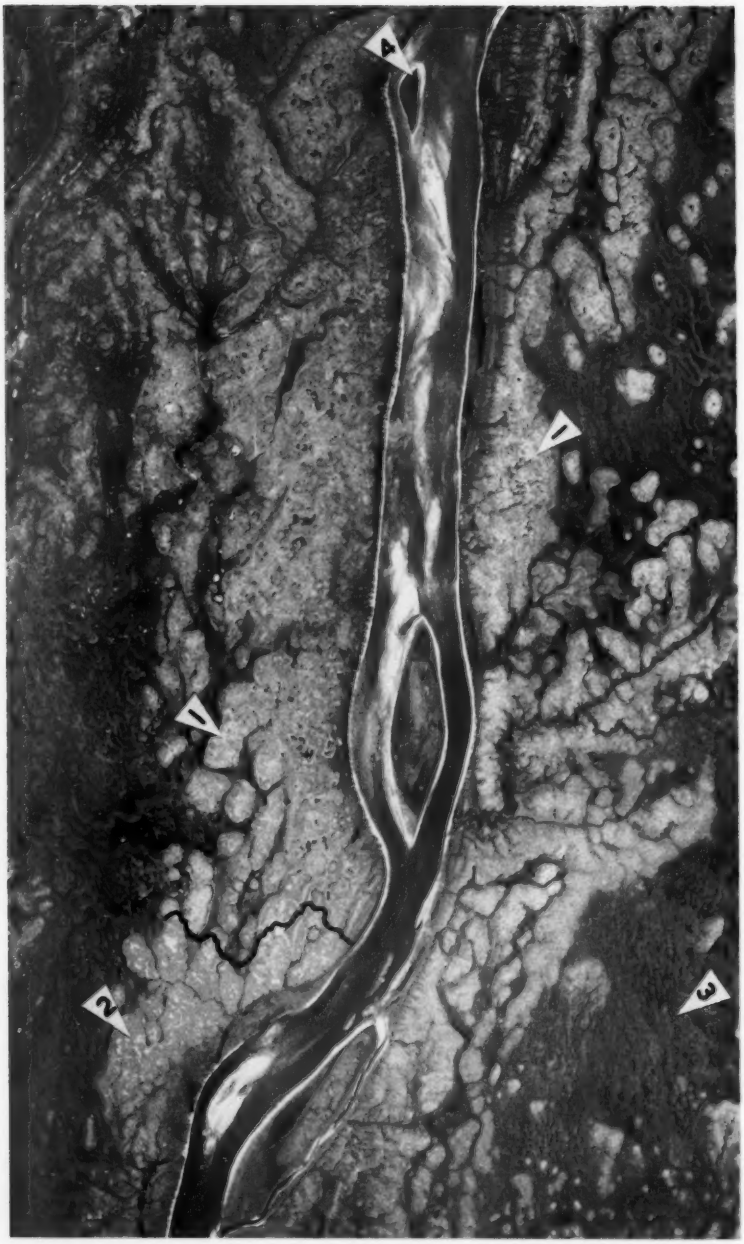
Using this map as a basis, the following general statement can be made concerning the vegetation of the area. On the most recently exposed marine and alluvial habitats there is a fairly consistent zonation of vegetation, from marsh, shrub, poplar forest, to white spruce forest. These zones are found mainly along river channels, on islands and banks, and on recent beaches. Inland the poorly drained, flat terrain is covered by a mantle of peat of varying thickness and nature. Slightly raised ground, especially old beaches and river levees, are covered by an open stand of stunted black spruce with a dense carpet of lichens covering the surface (Figs. 4 and 10). This category, named lichen muskeg (18), occupies approximately 35% of the land surface of the mapped area. Locally it has been burned and the spruce–lichen vegetation replaced by dense shrubs. Between these slightly raised areas of peat, the wetter, usually shallower peats are occupied by a fen vegetation in situations nearer the shorelines and rivers, and by palsa and string bog further inland on older sites. In general, the wetter fens lack tree cover and the less wet have an open stand of poorly grown *Larix*.

Picea glauca Forest

White spruce is confined here to substrata where the mineral layers are found not more than approximately 30 cm from the surface. Such conditions are found along the banks of rivers and streams, on river islands, and on raised beaches. Either because of their improved drainage, or because they are more youthful, these sites lack a thick mantle of peat. *Picea glauca* occurs as closed

FIG. 3. A portion of vertical aerial photograph A 14219-105 (scale 1:60,000), showing part of the Hayes River and the young flats between the Hayes and Nelson. Cover types visible are moss and lichen muskeg (A) often occurring on old beach lines, white spruce forest on alluvium (B), shrub on alluvium (C), larch fen (D), and shrub fen (E). (Photograph by courtesy of the Royal Canadian Air Force.)

Ritchie—Can. J. Botany



Ritchie—Can. J. Botany

stands, the trees showing good growth. There is evidence (25) that in this locale these stands have been influenced by man, who has used them for construction materials and fuel.

The largest continuous stands are found on some of the islands of the Lower Hayes River, but none exceeds 16 hectares and many are so fragmentary that they could not be mapped in Fig. 2. No stand examined was developed on a mature soil profile, and all the 'soils' examined consisted of an upper horizon of organic matter overlying the mineral material, usually a calcareous boulder clay or gravel. The organic layers are of fibrous to amorphous peat, 15 to 20 cm in depth. Permafrost was noted in two stands at 45 to 50 cm depth in late July. The largest white spruces recorded were 20 m in height, 15 cm d.b.h., at 200 years, but values of 15 m and 12 cm d.b.h. at 200 years are more typical.

The minor vegetation of these stands is relatively uniform, and the data from six stands presented in Table III is likely to be representative. There is a tall to medium shrub stratum of scattered individuals, never forming a continuous layer, the chief species being *Alnus rugosa*, *Salix cordifolia* var. *callicarpaea*, *Viburnum edule*, and *Shepherdia canadensis*. The ground vegetation has a conspicuous moss component, dominated by *Hylocomium splendens*. Low shrubs occurring frequently in all stands are *Salix myrtillifolia*, *S. vestita*, *Empetrum hermaphroditum*, *Vaccinium vitis-idaea* ssp. *minus*, and *Arctous alpina*. Herbaceous plants of common occurrence, though seldom covering large areas, are *Equisetum sylvaticum*, *Linnaea borealis* var. *americana*, *Mitella nuda*, *Habenaria obtusata*, *Pyrola minor*, and *Geocaulon lividum*.

Populus balsamifera Forest

In this area *Populus balsamifera* occurs in two types of community. Most commonly, it forms pure stands, more or less even-aged, growing on islands and floodplain gravels of the Hayes River (Fig. 2). Elsewhere, it occurs on older alluvium as small groups, or even singly, with *Picea glauca*, and usually the poplars are decadent with no signs of reproduction.

As Fig. 2 illustrates, those more recent river islands which bear poplar stands have a peripheral willow zone surrounding the central stand of *Populus*. Further up the river, similar islands have a central stand of white spruce with concentric zones of poplar and willow. Similarly, along certain streams there are zones of sedge at the water's edge, willow, balsam poplar, and white spruce on the summit of banks (Fig. 5). That is, in general balsam poplar occupies a zonal, and probably seral, position between a willow belt and the white spruce forest. The larger poplar stands on islands form pure stands with a dense canopy, no under shrubs, and very sparse ground vegetation. The soil is predominantly mineral, with very slight accumulation of organic material and no profile development. Usually it is a calcareous clay, locally with pebbles and rounded stones. The ground vegetation nowhere forms a continuous mat,

FIG. 4. A portion of vertical aerial photograph A 14220-174 (scale 1:60,000), showing part of Hayes River and the adjoining land. The cover types visible are lichen muskeg (1), shrub communities on previously burned-over lichen muskeg (2), palsa bog complexes with occasional "islands" of lichen muskeg (3), and poplar forest on an island (4).

TABLE III

A cover/sociability analysis of the ground vegetation of white spruce stands

Species	Stands 1	2	3	4	5	6
Hylocomium splendens	4.5	5.5	3.4	4.5	4.4	4.5
Vaccinium vitis-idaea ssp. *minus*	2.2	2.2	1.2	2.2	1.2	2.2
Equisetum sylvaticum	2.2	1.2	2.2	1.2	1.2	1.2
Arctous alpina	1.3	1.2	–	1.3	1.3	1.2
Empetrum hermaphroditum	1.4	1.2	1.2	–	1.3	1.3
Cladonia rangiferina	1.2	1.1	1.1	1.2	+	1.1
Salix vestita	1.1	1.1	+	1.1	+	+
Linnaea borealis var. *americana*	1.1	2.2	+	1.1	–	+
Pyrola minor	+	1.1	+	–	1.2	+
Tomenthypnum nitens	1.1	+	+	1.1	–	–
Tetraplodon angustatus	1.3	++	1.1	1.1	+	–
Mertensia paniculata	1.1	–	–	–	1.1	–
Petasites palmatus	+	–	+	–	1.1	+
Aulacomnium palustre	1.3	–	–	–	1.1	–
Carex concinna	1.1	+	–	–	–	–
Pyrola secunda	1.1	+	–	+	–	+
Cypripedium passerinum	+	–	+	+	–	–
Habenaria obtusata	+	+	+	–	–	+
Cladonia amaurocraea	+	–	–	+	–	+
Ledum groenlandicum	1.1	–	–	–	+	–
Listera auriculata	+	–	–	–	–	+

Species	Stands 1	2	3	4	5	6
Cladonia gracilis	+	–	+	–	–	–
Epilobium angustifolium	+	–	–	–	–	–
Mitella nuda	1.1	+	–	+	+	+
Bistorta vivipara	+	–	–	+	–	–
Peltigera aphthosa	+	–	+	–	–	+
Splachnum ovatum	+	–	+	–	–	–
Drepanocladus vernicosus	+	–	+	+	–	–
Isopterygium pulchellum	–	+	–	–	–	–
Drepanocladus exannulatus	–	+	–	–	+	–
Dicranum bergeri	–	+	–	–	–	–
Rosa acicularis	–	+	–	–	–	+
Ribes triste	–	–	+	–	–	–
Dicranum groenlandicum	–	–	–	+	–	–
Peltigera polydactyla	–	–	–	+	–	–
Lycopodium annotinum var. *pungens*	–	–	–	–	+	–
Abietinella abietina	–	–	–	–	+	–
Cladonia multiformis	–	–	–	–	–	+
Equisetum scorpoides	–	–	–	–	–	+
Rubus acaule	–	–	–	–	–	+
Tofieldia pusilla	–	–	–	–	–	+

being made up of scattered individuals of the following species: *Equisetum arvense*, *Achillea borealis*, *Thalictrum venulosum*, *Arenaria laterifolia*, *Fragaria virginia*, *Epilobium angustifolium*, and *Actaea rubra*. Two stands of this type were noted on floodplain sites with a subsidiary stratum of young trees of *Picea glauca*, indicating local replacement of poplar by spruce. The other type of poplar site, where it is remnant and sporadic, was too poorly represented in the area to permit more than general observations. The substratum indicated an older site than the young dense stands, with 10 to 15 cm of organic matter —amorphous and black—over the mineral material. The ground vegetation is typical of white spruce stands (Table III), with the commonest species, *Equisetum sylvaticum*, *Salix vestita*, *Mitella nuda*, *Ribes glandulosa*, *Rosa acicularis*, *Hylocomium splendens*, *Carex leptalea*, *C. brunnescens*, *Linnaea borealis* var. *americana*, and *Rubus pubescens*.

It is of interest that Bell (4), on the map accompanying his report, shows the lower limit of poplar trees on Hayes River as an island 1.5 km above the present limit. It is likely that this represents an actual change in time, since Bell's observations and map were made with accuracy. Bell ((4) p. 25 cc) suggests this possibility of succession himself and discusses the progressive emergence of islands as the "waters of Hudson Bay receded". This subject, and the general question of succession of vegetation, are discussed at the end of this paper.

Shrub Vegetation

Continuous tracts of shrub vegetation are found on recently exposed flats, on river islands, and along stream banks. On the low flats, along the shores of the estuaries and the headland between them (Marsh Point), there are wide belts of scrub, abutting on marsh zones nearer the present shoreline and forested zones inland. The chief species of these young sites is *Salix brachycarpa*, associated with *S. cordifolia* var. *callicarpaea* and *S. planifolia*. In openings along the invading fringe of this scrub belt there are patches of meadow with a mixture of species, all recorded below in the section on marsh vegetation. In small depressions with wet substrata *Ranunculus gmelinii*, *Senecio congestus*, *Triglochin maritima*, and *Rumex arcticus* are common. *Bryum pseudotriquetrum* occurs frequently, forming compact cushions in association with *Campylium polygamum*, and *Drepanocladus aduncus* var. *polycarpus* f. *gracilescens*.

The youngest river islands (Fig. 2) are covered by a more or less continuous scrub, the commonest species being *Salix rigida*, *S. planifolia*, and *S. cordifolia*. This zone extends as far down as the upper limit of spring flood, where water and ice action prevent the establishment of any perennial or woody plants. On the smaller islands there is evidence that the spring floods inundate the entire island, the water and ice removing much of the vegetation and depositing clay and silt. On older islands further from the mouth of the river, the willows form a peripheral zone, in some cases separated from the central spruce forest zone by a poplar zone.

In contrast to the steep, denuded banks of the Hayes and Nelson rivers where large volumes of ice-charged spring waters scar the banks annually, small streams (e.g. French Creek, Fig. 5) have stable, vegetated banks. There is a fairly constant zonation of vegetation along these small streams, consisting of a narrow discontinuous sedge fringe, a broad belt of shrubs, and a white spruce community on the upper bank slopes and summits. In some instances there is a narrow poplar zone between the shrub and spruce zones. The shrub zone is dominated by *Salix planifolia* and *Alnus rugosa* ssp. *americana* with sporadic occurrences of *Salix cordifolia* and *S. myrtillifolia*. Under the willow stratum, which reaches 3 m in height, there is a sparse vegetation of low shrubs and herbs, a representative sample of which is given in the following analysis:

LOW SHRUB LAYER:
Viburnum edule, *Ribes glandulosum*, *Rosa acicularis*, and *Rubus pubescens*.
HERB AND MOSS LAYER (cover values):

Anemone richardsonii	– 2	*Calliergon cordifolium*	+
Equisetum sylvaticum	– 2	*Listera auriculata*	– +
Achillea millefolium	– 1	*Thalictrum sparsiflorum* var. *richardsonii*	– +
Corallorhiza trifida	– 1	*Mertensia paniculata*	– +
Epilobium angustifolium	1	*Hypnum lindbergii*	– 1
Mitella nuda	– 1	*Calamagrostis canadensis*	– 1
Fragaria virginiana	– 1	*Carex disperma*	– +
Galium trifidum	– 1	*Campylium chrysophyllum*	– +
Drepanocladus vernicosus	– +		

Marsh

On the most recently exposed flats of the estuary and shores, a small number of plants form an outer zone of salt marsh vegetation. These plants are mainly rhizomatous, forming more or less pure patches. The chief species are *Eleocharis palustris*, *Hippuris tetraphylla*, *Colpodium fulvum*, and *Calamagrostis deschampsioides*. Immediately above this zone there is a zone dominated by sedges, occupying the wet mud above the normal high tide level. The dominant plant at Marsh Point is *Carex palacaea*, associated with *C. miliaris*, *C. aquatilis*, *Senecio congestus*, *Rumex arcticus*, *Potentilla egedii* var. *groenlandica*, and *Calamagrostis deschampsioides*. This zone varies from 10 to 30 m in width. It abuts onto a broad meadow or dry marsh, dominated by grasses and herbs. The tract of dry marsh at Marsh Point was sampled and yielded the following cover/sociability estimates:

Dupontia fischeri var. *psilocantha*	– 3.4	*Castilleja raupii*	– +
Hierochloe odorata	– 1.3	*Saxifraga hirculus*	– +
Chrysanthemum arcticum	– 1.1	*Parnassia multiseta*	– 1.1
Potentilla egedii var. *groenlandica*	– 1.1	*Petasites sagittatus*	– +
Primula egaliksensis	– 1.1	*Hordeum jubatum*	– 1.2
Calamagrostis deschampsioides	– 1.3	*Ranunculus gmelinii*	– +
Lomatogonium rotatum	– +	*Bryum pseudotriquetrum*	– +
Festuca rubra	– 1.2	*Campylium polygamum*	– +
Puccinellia distans	– 1.2	*Drepanocladus aduncus* var. *polycarpus* f. *gracilescens*	– +
Plantago juncoides	– 1.1		

There is a broad transition between this meadow zone and the adjacent shrub zone on older substrata.

TABLE IV

A cover/sociability analysis of stands of lichen muskeg

Species	Stands					Species	Stands				
	1	2	3	4	5		1	2	3	4	5
Ledum groenlandicum	4.5	4.5	4.5	4.5	4.5	*Tetraplodon angustatus*	+	–	–	+	+
Cladonia alpestris	1.2	1.1	1.3	1.3	1.3	*Eriophorum spissum*	1.2				1.2
C. rangiferina	4.5	3.4	3.5	4.5	3.5	*Peltigera aphthosa*	+	–	++	+	+
Vaccinium uliginosum	2.2	1.2	1.1	2.2	1.2	*Aulacomnium palustre*	1.1	+	1.1	1.1	1.1
V. vitis-idaea ssp. *minus*	2.3	1.2	1.3	1.2	2.2	*Kalmia polifolia*	++	–	–	–	–
Polytrichum juniperinum	2.3	1.3	2.2	1.2	1.3	*Calamagrostis canadensis*	++	1.1	+	++	+
Sphagnum fuscum	2.1	1.1	+	1.2	1.2	*Pleurozium schreberi*	1.1	+	–	++	–
Salix myrtillifolia	1.2	–	1.2	–	1.2	*Arctous alpina*	+	–	–	–	–
Empetrum hermaphroditum	2.2	1.3	1.2	+	1.2	*Sphagnum capillaceum*	1.1	1.1	+	+	–
Rubus chamaemorus	1.1	1.1	+	1.1	1.1	*Peltigera canina*	–	+	–	–	–
Smilacina trifolia	+	–	1.1	+	1.1	*Ersgeron hyssopifolius*	–	–	+	–	–
Ledum decumbens	2.1	2.1	1.1	–	1.1	*Ranunculus lapponicus*	–	–	–	++	–
Dicranum bergeri	1.1	+	+	–	+	*Sphagnum girgensohnii*	–	–	–	++	–
Equisetum scirpoides	+	–	++	–	–	*Cladonia coccifera*	–	–	–	+	–
Tetraplodon mnioides	1.1	+	–	–	+	*C. deformis*	–	–	–	–	+

Minor Communities

Local discontinuities in habitat are often reflected in the occurrence of fragmentary associations of plants which differ from the general, more or less continuous cover types that prevail. In this area, the unstable river banks and floodplains provide such niches, where transient assemblages of plants are found, seldom forming a closed sward. Two types of such river habitat can be recognized—the steep, unstable clay banks of the river trench, and occasional slip-off slopes where pebbles and water-worked boulders have been exposed.

The former site is usually wet, as water oozes from the overlying peat and clay. The slope is steep (Fig. 6) and many upper, ice-scarred slopes are free of any plant growth. Elsewhere, pioneer species become established and form a loose open community which is subject to considerable erosion, but which shows floristic and structural constancy in the area. On the lower, less steep surfaces near the normal summer water level, certain species with efficient means of vegetative propagation form clones, particularly *Equisetum palustre*, *Triglochin palustris*, *Juncus balticus* var. *littoralis*, *Eriophorum scheuchzeri*, and *Hippuris vulgaris*. Situations where there is considerable lateral seepage of water down the bank bear a richer community, with the following species (cover/sociability values given for one sampled community):

Equisetum palustre	– 1.3	*Castilleja raupii*	– 1.1
Philonotis fontana	– 1.4	*Juncus castaneus*	– 1.3
Hypnum lindbergii	– 1.4	*Rumex arcticus*	– +
Drepanocladus uncinatus	– 1.3	*Cypripedium passerinum*	– 1.1
Primula stricta	– 1.1	*Triglochin palustris*	– 1.3
Pinguicula vulgaris	– 1.1	*Drepanocladus vernicosus*	– 1.2
Bryoerythrophyllum recurvirostrum	– 1.3	*Carex aurea*	– 1.3
Hippuris vulgaris	– 1.2	*Erysimum cheiranthoides*	– +
Primula egaliksensis	– 1.1	*Timmia megapolitana*	– +

Locally, where erosion has formed a large gully in the bank with a landslide, the gentler slopes are colonized by *Salix cordifolia* var. *callicarpaea* and *Betula glandulosa*.

The second type considered here is the relatively rich group of plants found on calcareous mineral substrata of certain slip-off slopes along the Hayes River, especially along the stretches of river about 50 km from the mouth. The substratum consists of rounded pebbles and boulders with calcareous clay. The vegetation is open, made up of colonizing species which occur singly or tufted in groups (the latter mainly grasses). A cover/sociability analysis was not made and they are listed here in order of approximate relative abundance: *Epilobium latifolium*, *Poa alpina*, *Trisetum spicatum* var. *molle*, *Artemisia*

FIG. 5. French Creek, 2 km from its junction with Hayes River. On the opposite bank there are zones of *Carex*, *Salix planifolia*, *Populus balsamifera*, and *Picea glauca*.

FIG. 6. Hayes River from a small island about 8 km from the mouth. On the opposite shore the steep clay banks are visible, capped by a mantle of peat bearing continuous moss muskeg vegetation.

FIG. 7. Salt marsh vegetation at Marsh Point. At the left side, beside the figure, is a dense stand of *Eleocharis palustris* with *Hippuris tetraphylla*. The remainder of the marsh vegetation in view is dominated by *Carex palacea* and *C. miliaris*.

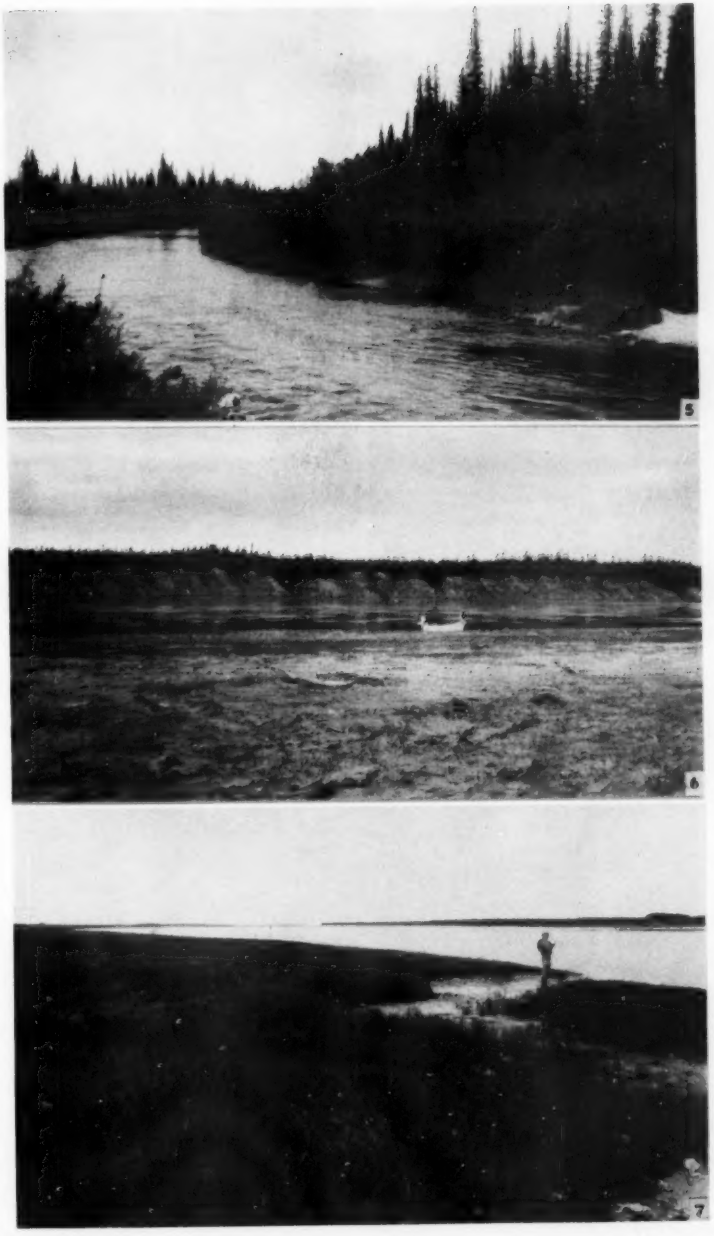


PLATE IV

canadensis, *Arnica alpina* ssp. *attenuata*, *Elymus innovatus*, *Astragalus alpinus*, *Hedysarum mackenzii*, *Galium septentrionale*, *Oxytropsis campestris* var. *varians*, *Elaeagnus commutata*, *Potentilla fruticosa*, *Anemone parviflorum*, *Carex scirpoidea*, *Smilacina stellata*, *Potentilla anserina*, *Anemone multifida*, *Juniperus communis*, *Achillea millefolium*, *Rosa acicularis*, *Habenaria viridis* var. *bracteata*, and *Allium schoenoprasum* var. *Sibiricum*.

It is in open, unstable situations of this nature that the greatest floristic diversity and interest is to be found in this region.

Lichen Muskeg

This remarkable vegetation type is found on relatively deep peat on areas raised slightly above the general level of the land. The depth of the peat is 0.75 m to 1.50 m. As Fig. 2 suggests, lichen muskeg is well developed on river levees and on older, weathered beaches. The substratum consists of thick layers of coarse, red-brown *Sphagnum* peat, with one or two alternating bands of fibrous, dark-brown heath peat. The upper 5 cm are of black amorphous peat with recognizable lichen and heath remains in the top 2 cm. In late July the depth of the active layer was 0.50 m to 0.75 m.

The tree layer of the vegetation is composed entirely of well-spaced individuals of *Picea mariana*, usually with a bushy-base habit. The average tree density of six sampled stands was five per 100 sq. m. The trees seldom exceed 10 m in height at 200 years of age, the average values for the stands examined being 7.5 m in height, 9 cm d.b.h. at 170 years. A very sparse stratum of tall shrubs occurs occasionally, the main species being *Salix arbusculoides*, *S. planifolia*, and *Betula glandulosa*.

The ground vegetation is dominated by *Ledum groenlandicum* and a continuous carpet of *Cladonia* species of lichen, mainly *C. rangiferina* (Fig. 10). The *Ledum* and *Cladonia* components occupy approximately equal areas. Associated plants are *Vaccinium uliginosum*, *V. vitis-idaea* ssp. *minus*, *Polytrichum juniperinum*, *Empetrum hermaphroditum*, *Ledum decumbens*, *Rubus chamaemorus*, and *Sphagnum fuscum*. A more complete survey of the community can be gained from Table IV.

It was observed in several localities that the fruticose *Cladonia* appeared to be replacing colonies of *Polytrichum juniperinum* and *Sphagnum fuscum*, and it seems likely from the nature of the peat strata that this general successional trend has prevailed on a large scale.

Small depressions in the peat are wet and usually dominated by *Drepanocladus aduncus*, *D. vernicosus*, and *Bryum pseudotriquetrum*. *Eriophorum spissum* is common in these situations.

FIG. 8. The interior of a moss muskeg stand showing the abundance of *Ledum groenlandicum* under *Picea mariana*.

FIG. 9. The interior of a mature stand of *Picea glauca* on alluvium. The shrub is *Alnus rugosa* and the ground vegetation is dominated by *Hylocomium splendens*.

FIG. 10. In the foreground, the edge of a levee with lichen muskeg, dominated by *Ledum groenlandicum*, *Cladonia rangiferina*, and *Picea mariana*. In the distance are tall *Picea glauca* along the banks of the adjacent stream.

TABLE V

A cover/sociability analysis of stands of moss muskeg

Species	Stands 1	2	3	4	5
Ledum groenlandicum	5.5	4.5	5.5	4.5	4.5
Empetrum hermaphroditum	2.4	1.4	1.4	3.4	2.4
Rubus chamaemorus	2.1	2.1	1.1	1.1	3.1
Hylocomium splendens	3.5	2.5	2.5	1.5	3.5
Vaccinium vitis-idaea ssp. *minus*	2.3	3.5	1.3	1.3	1.3
V uliginosum	2.2	2.1	1.2	1.2	1.2
Sphagnum fuscum	1.4	2.4	1.4	+	1.4
Ledum decumbens	1.1	1.1	2.1	+	1.1
Cladonia rangiferina	1.3	1.4	+	1.3	1.3
Peltigera aphthosa	2.2	1.1	1.1	+	1.1
Aulacomnium palustre	1.1	+	+	+	+
Sphagnum capillaceum	1.1	1.1	+	−	+
Cladonia rangiformis	+	1.1	+	−	−
Peltigera canina	1.1	−	1.1	−	+
Pinguicula villosa	+	+	−	+	−
Geocaulon lividum	1.1	−	+	+	+

Species	Stands 1	2	3	4	5
Pleurozium schreberi	1.1	1.1	+	1.1	+
Tomenthypnum nitens	1.1	+	−	−	−
Ptilium crista-castrensis	+	−	+	−	−
Orchis rotundifolia	+	−	−	−	−
Corallorhiza trifida	−	+	−	−	−
Ranunculus lapponicus	−	+	−	−	+
Mnium andrewsianum	−	1.1	+	+	+
Calliergon stramineum	−	1.1	1.1	+	1.1
Habenaria obtusata	−	−	+	−	−
Arctous alpina	−	−	+	−	+
Eriophorum spissum	−	−	+	−	−
Carex vaginata	−	−	−	+	−
Equisetum sylvaticum	−	−	−	+	−
Habenaria hyperborea	−	−	−	−	+
Viola palustris	−	−	−	−	+
Luzula parviflora	−	−	−	−	+

Moss Muskeg

Moss muskeg seldom forms large continuous areas here, as it does elsewhere in the Lowlands, and with the exception of the patches on the north bank of Hayes River (Fig. 2), it is represented by small patches, seldom more than 2 hectares. With respect to floristic, structural, and ecological features it is very similar to lichen muskeg. It occurs on the deeper moss peats (0.75–1.50 m), but apparently they are wetter than the substrata of lichen muskegs. The main floristic difference is the prevalence of mosses in the ground vegetation of moss muskeg, but *Ledum groenlandicum* is present in about the same relative amount as in lichen muskeg (Fig. 8). The trees are more dense than in lichen muskeg, averaging 12 per 100 sq. m. The values recorded for trees of 180 to 200 years were 10 m in height and 17 cm d.b.h.

There is no tall–medium shrub stratum, but *Ledum groenlandicum* dominates the minor vegetation, associated with *Hylocomium splendens*, *Empetrum hermaphroditum*, *Rubus chamaemorus*, *Vaccinium uliginosum*, *Sphagnum fuscum*, and *Aulacomnium palustre*. The analysis in Table V provides a more detailed picture of the community.

Depressions are common in muskegs, in which the water table is near the surface, and they are dominated by the following mosses in variable proportions: *Calliergon stramineum*, *Mnium andrewsianum*, *Drepanocladus aduncus*, *Hypnum lindbergii*, and *Bryum pseudotriquetrum*.

Palsa and String Bog

Only superficial examination was made of these less accessible types, and no quantitative analyses were attempted. Palsa bogs are found on older sites where more than 1 m of peat has accumulated. Their characteristic feature is an irregular mounded surface, with the mounds varying from 3 m to 15 m in diameter, and in height generally 1.0 to 1.5 m above the level of the surrounding peat. The surface usually lacks trees or has only stunted black spruce. The ground vegetation resembles that of lichen muskeg, but there is more *Ledum groenlandicum* and less *Cladonia*. Other common species are *Vaccinium uliginosum*, *V. vitis-idaea* ssp. *minus*, *Sphagnum fuscum*, *Aulacomnium palustre*, *Rubus chamaemorus*, and *Empetrum hermaphroditum*. Between the mounds there are areas of shallow open water or wet bog, the chief species in these situations being *Menyanthes trifolia*, *Eriophorum spissum*, *Carex rostrata*, *Drepanocladus uncinatus*, *Calliergon stramineum*, and *Sphagnum capillaceum*.

Elsewhere, the raised peat masses are arranged linearly in parallel ridges, with wet bog between. Poorly grown spruce and larch occur on the ridges. There was no opportunity to examine this type in the field. In passing, it is relevant to note that larch fen sometimes shows a parallel ridge pattern.

Secondary Bog Communities

In the map (Fig. 2) there are four areas mapped where the original lichen muskeg has been destroyed by fire and secondary vegetation has replaced it. One example was examined, on the Hayes River, and the vegetation was found to consist of a shrub layer dominated by *Salix planifolia* with a few

individuals of *S. arbusculoides* and *Betula glandulosa*. The ground vegetation consists of the following species, in approximate order of relative abundance: *Calamagrostis canadensis*, *Deschampsia caespitosa* var. *littoralis*, *Achillea millefolium*, *Epilobium angustifolium*, *Erysimum cheiranthoides*, *Stellaria longipes*, *Carex vaginata*, and *Potentilla anserina*.

Larch Fen

The cover type designated larch fen is distinguished primarily by floristic and structural features, but it appears also to have considerable ecological distinctness. It consists of an open stand of *Larix laricina*, generally poorly grown trees, with a discontinuous shrub stratum of *Betula glandulosa* and *Salix*, and a ground vegetation complex of sedges, herbs, and mosses. As in all the bog and fen communities described here, mosses form the largest component of the ground vegetation in terms of cover, and in fens they show great floristic diversity.

In this particular region, fen vegetation is developed on shallow (less than 1 m) poorly drained peat overlying calcareous clay, and all large tracts of fen occur near the coasts and rivers (Fig. 2). The area immediately south of the mouth of the Hayes River (Fig. 2) shows the characteristic disposition of fen and bog communities in relation to drainage channels. The slightly elevated (levees) strips along the streams bear deep (greater than 1 m) *Sphagnum* peats with lichen muskeg and the flat interfluves with wetter, shallower peat are occupied by fen, usually larch fen. The peaty substratum of larch fens is neutral or basic in reaction (pH values range from 7.0 to 8.2) with the exception of occasional mounds of *Sphagnum* (generally *S. warnstorfianum*) with pH values of about 5. The following data were collected from specimens of *Larix*, and they illustrate the retarded nature of the growth: 2 m height, 7 cm d.b.h. at 74 years; 4.5 m height, 9 cm d.b.h. at 154 years; 9 m height, 15 cm d.b.h. at +300 years. The density of individuals of larch taller than 1 m is approximately eight per 100 sq. m.

The shrub layer is open, up to 1.5 m in height, made up primarily of *Betula glandulosa* and smaller amounts of *Salix pedicellaris* var. *hypoglauca*.

The minor vegetation reveals the greatest floristic diversity of any of the continuous, relatively stable vegetation types in the area. It forms a rough mosaic of two components which can be readily distinguished on casual observation. One, occupying 60–70% of the total fen area, is dominated by sedges, herbs, and mosses, the last consisting particularly of members of the Hypnales. The other consists of low peat mounds carpeted by pure patches of *Tomenthypnum nitens* and/or *Sphagnum warnstorfianum*, associated constantly with *Oxycoccus microcarpus*, *Andromeda polifolia*, *Vaccinium vitis-idaea* ssp. *minus*, *Aulacomnium palustre*, and *Smilacina trifolia*.

The sedge–herb–moss unit is in the form of a loose reticulum, with the mounds occurring as "islands" of variable size and frequency. The most consistently occurring species in the sedge–herb–moss mat are (in approximate order of relative abundance): *Carex chordorrhiza*, *C. diandra*, *Drepanocladus vernicosus*, *Calliergon stramineum*, *Carex paupercula*, *Paludella squarrosa*,

TABLE VI

A cover/sociability analysis of the ground vegetation of larch fen stands

Species	Stands				Species	Stands			
	1	2	3	4		1	2	3	4
					(*a*) Low peat mounds				
Sphagnum warnstorfianum	4.5	3.5	4.5	4.5	*Smilacina trifolia*	1.1	+	1.1	1.1
Aulacomnium palustre	2.4	1.4	2.4	2.4	*Equisetum sylvaticum*	1.1	–	++	1.1
Tomenthypnum nitens	3.4	2.4	3.4	1.4	*Empetrum hermaphrodium*	1.3	++	–	++
Hylocomium splendens	1.4	1.4	2.4	–	*Rubus chamaemorus*	++	++	–	++
Andromeda polifolia	1.2	++	1.2	1.2	*Helodium blandowii*	+++	1.1	+	–
Vaccinium vitis-idaea ssp. *minus*	1.2	++	1.2	1.2	*Salix myrtillifolia*	++	–	–	+++
Oxycoccus microcarpus	2.2	1.2	+	1.2	*Galium labradoricum*	–	+	–	+++
					Cladonia rangiferina	–	1.1	+	
					(*b*) Sedge–herb–moss mat				
Carex chordorrhiza	2.2	3.2	2.2	2.1	*Corallorhiza trifida*	+++	–	+	++
C. diandra	3.2	3.1	3.1	2.2	*Listera cordata*	+++	–	–	++
Drepanocladus vernicosus	4.4	2.4	2.4	1.4	*Aulacomnium palustre*	+++	1.2	1.1	1.1
Calliergon stramineum	2.3	2.4	1.4	2.4	*Mnium affine*	+++	+	+	+
Carex paupercula	1.2	1.2	+	1.2	*Comarum palustre*	++	1.2	1.1	1.1
Paludella squarrosa	1.3	1.2	1.3	1.3	*Menyanthes trifolia*	1.1	+	1.1	1.1
Meesia triquetra	1.2	1.1	1.1	1.2	*Petasites sagittatus*	2.2	–	1.2	+
Bryum pseudotriquetrum	1.1	1.1	1.1	1.1	*Caltha palustris*	1.1	1.1	1.1	1.1
Mnium andrewsianum	1.1	1.1	1.1	1.1	*Listera auriculata*	+	–	+	++
Triglochin maritima	1.1	1.2	1.1	1.1	*Pyrola minor*			–	–
Eriophorum viridi-carafum	+	1.1	1.2	1.1	*Kraeria glacialis*	–	–	–	–
Habenaria hyperborea	+	1.1	+	+	*Drepanocladus revolvens*	–	1.3	1.2	1.2
Tomenthypnum nitens	1.3	1.1	++	1.3	*D. uncinatus*	1.4	+	1.3	1.3
Carex leptalea	1.1	1.1	1.1	1.1	*Calliergon giganteus*	1.3	1.4	1.4	1.2
C. neurochlaena	+	–	1.1	+	*Cardamine pratensis* var. *angustifolia*	–	+	+	+

Meesia triquetra, *Bryum pseudotriquetrum*, *Mnium andrewsianum*, *Triglochin maritima*, *Eriophorum viridi-carinatum*, and *Habenaria hyperborea*. The species of *Calliergon*, *Paludella*, *Meesia*, *Mnium*, and *Bryum* grow in close association, forming a mixed mat of constant structure and composition. In the wettest depressions, where the water table is near the surface, the following species form local pure patches: *Drepanocladus revolvens*, *D. uncinatus*, *Calliergon giganteum*, and *Drepanocladus exannulatus*. A complete cover/sociability analysis of the complex is provided in Table VI, *a* and *b*, but it is clear that more refined methods of quantitative analysis would enable a more precise and empirical assessment of the nature of the apparent pattern and associations of species.

Shrub Fen

The floristic differences between larch and shrub fen are negligible. In the microphanerophytic layer, the latter lacks *Larix* (except rarely) and is dominated by *Salix cordifolia*, *S. brachycarpa*, and *Betula glandulosa*. In ground vegetation, the *Sphagnum* mound facies (Table VI, *a*) described above for larch fen is absent or poorly developed, and the ground vegetation is dominated by the sedge–herb–moss complex. Floristically and structurally it is identical with that of larch fens. Generally, the substratum is wetter, often with the water table at the surface of the peat, and the depth of peat is less than in larch fens.

Concluding Comments

Some attempt has been made to answer those questions which form the basis of a primary vegetation survey (in the sense of Cain and Castro (5)), namely: What kinds of vegetation cover are encountered in the area? Where, topographically, do they occur? What are their floristic and structural features? It is remarkable that although the area studied here has been visited by several botanists and others since the times of the expeditions of Franklin (see ref. 15), any adequate recorded observations on vegetation have been highly unrepresentative. This leads one to repeat the simple point made by Hare (11) that much of our knowledge of the northern vegetation of Canada is based on the reports of scientists whose summer travels were exclusively by canoe routes, and who seldom gathered any information on the nature of the interfluves. For example, in the Lower Hayes River no mention is made in the reports of Bell (3, 4) and Scoggan (21) of what we call here lichen muskeg, although Fig. 2 indicates its overwhelming preponderance as a cover type. This lack of representative information is particularly marked in the Hudson Bay Lowlands, and there is need for many more primary surveys, using as a tool the vertical aerial photograph.

A further question posed in a primary vegetation survey concerns any possible ecological or seral relationships. Here an objective answer is not possible, and any comments, such as the following, are based on personal judgement.

On the younger alluvium, particularly river islands and shores, it is likely that the zonation within older islands and the general progression from younger to older islands of shrub, poplar, and spruce communities represents a seral phenomenon. The interesting observations made by Bell (4) in 1879 enable a fairly precise comparison to be made between the vegetation of river islands at points in time 80 years apart. He writes, for example, that ((4) p. 25 cc) "Four Mile Island has become overgrown with small poplars, while it is evident that at no very distant period Six Mile Island formed two islands which are now covered with full-sized trees, while the old channel between them now supports a growth of tall bushes." He shows the lower limit of trees to be Four Mile Island. Today, Four Mile Island has also spruce, and islands 1.5 and 3 m respectively further down the river have small stands of young poplar. Likewise, one obtains a clue to the succession by travelling up the lower stretch of the river. On the bank, a rough profile view of the terrain is obtained, and the occurrence of raised beaches can be detected easily. On these slight ridges in the lowest stretches of the river the first poplars appear, in a vegetation which is predominantly willows. Further up, white spruce and some larch accompany the poplar, but the larch seldom grows on the actual ridges. Then the poplar drops out and white spruce prevails.

On poorly drained sites there has been an accumulation of peat, and it is suggested as a basis for further consideration that the general progression on wet land has been from marsh vegetation through shrub fen, larch fen, to bog, culminating probably in the prevalent lichen muskeg. The evidence is fairly clear (10, 4) that the continued rising of the land about Hudson Bay is making available new habitats for colonization, and from the shores inland the general zonation of vegetation probably reflects accurately the sequence of colonization (17). Accordingly, one might interpret the prevalence of large tracts of fen on younger sites and its scarcity further inland (Fig. 2) as an indication of the expected sequence of fen to bog, with the earliest stages being marsh and scrub. Within fens there is evidence that *Sphagnum* becomes established and initiates the accumulation of acid peat which changes the habitat and vegetation to a bog type. Accompanying this development of bog there is probably a progressive impoverishment of flora, with the only inland sites of floristic diversity and richness being unstable river habitats.

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SYNERGISM BETWEEN PRATYLENCHUS PENETRANS (COBB) AND VERTICILLIUM ALBO-ATRUM R. & B. IN EGGPLANT WILT[1]

C. D. McKeen and W. B. Mountain

Abstract

Studies conducted in steam-sterilized soil inoculated with *Verticillium albo-atrum* and *Pratylenchus penetrans* demonstrated a synergistic relationship between fungus and nematode. At low and intermediate levels of *Verticillium* inoculum, the nematode increased wilt and the increase may be a function of the number of nematodes present. Except at the high inoculum levels of *Verticillium*, significantly larger numbers of *P. penetrans* occurred in eggplant roots in the presence than in the absence of the fungus. The nematode alone had no adverse effect on root condition or growth of eggplant.

Introduction

Wilt of eggplant (*Solanum melongena* L.), caused by the soil-borne fungus *Verticillium albo-atrum* Reinke and Berth., is known to be a serious disease wherever the crop is extensively grown. On the light sandy soils of the Harrow–Leamington area of southwestern Ontario the disease has caused heavy to complete losses in yield in recent years. In many instances heavy infestations of the soil have undoubtedly resulted from growing eggplant too frequently along with such other susceptible solanaceous hosts as tomato and potato.

In the absence of wilt-resistant varieties it has been necessary to resort to other means of possible control. In 1957, the senior author studied the applicability of certain agricultural chemicals to field plots for wilt control. He found that soil fumigation with Dowfume MC-2 (98% methyl bromide plus 2% chloropicrin) provided satisfactory disease control for a single season. In 1958 and 1959, the nematicides Shell DD (dichloropropane dichloropropene) and Telone (mixed dichloropropenes) were found to delay the initial but not the later incidence of wilt.

In 1958, toward the end of the growing season the second author found an appreciable population of the root-lesion nematode, *Pratylenchus penetrans* (Cobb), in the roots of wilted and of non-wilted eggplant. In roots from sites where methyl bromide had been applied, exceedingly low nematode counts were obtained. In roots from soils treated with Shell DD and Telone somewhat larger populations of the nematode were recorded, but these populations were appreciably lower than those observed in the roots of plants from untreated areas.

Because of the evidence that nematodes might be playing a role in the etiology of *Verticillium* wilt of eggplant, growth chamber studies were conducted to explore this possibility. This paper reports the findings of this research.

[1]Manuscript received May 18, 1960.
Contribution No. 18, from the Research Station, Research Branch, Canada Department of Agriculture, Harrow, Ontario.

Literature Review

The concept that nematodes may interact with fungi in the etiology of root diseases of plants is not new, and during the last 20 years a number of publications have dealt with this subject. In almost all cases, the fungus involved has been a representative of the genus *Fusarium* and a synergistic relation between the fungus and nematodes, especially species of the root knot nematode (*Meloidogyne*), appears to have been well established (4). Other fungi with which nematodes have been implicated in plant diseases include *Phytophthora* (10), *Cylindrocarpon* (3, 13), *Rhizoctonia* (2, 11, 15), and *Dilophospora* (1). As to the role of nematodes in *Verticillium* wilt little information is available. McClellan *et al.* (5) were unable to demonstrate any interrelation between root knot nematodes and *Verticillium* sp. in wilt of cotton in California. Parker (8), having recently reviewed relevant literature, found some evidence therein which suggests that damage to plants by root knot nematodes and *Verticillium* spp. may be additive, but there has been no evidence of synergism, as in the case of nematodes and *Fusarium* wilt.

Root lesion nematodes (*Pratylenchus* spp.) have only rarely been implicated in fungus diseases of plants and synergism has not been demonstrated. Smith (14) observed that varieties of cotton normally resistant to *Fusarium* wilt showed evidence of the disease when *Pratylenchus* sp. was the predominant plant parasitic nematode present. In Holland, *Cylindrocarpon radicicola* was associated with *Pratylenchus penetrans* in a root rot of narcissus (13). A root rot of winter wheat occurring in Ontario appears to be caused by the additive injurious effects of *Pratylenchus minyus* and *Rhizoctonia solani* (2).

Materials and Methods

Essentially, the work reported herein consists of a series of tests in which decreasing amounts of *Verticillium* inoculum were added to steam-sterilized soil, and changes in the incidence of wilt were observed in the presence and absence of *Pratylenchus penetrans*.

Verticillium albo-atrum used in these studies was isolated from wilted eggplant in July, 1957. The isolate produced an abundance of carbonaceous microsclerotia on potato dextrose agar (PDA) and stock cultures were maintained on this medium. Inoculum for all experiments consisted of microsclerotial fragments obtained as follows: Petri plates of 100-ml diameter containing 16 ml PDA were inoculated by streaking with microsclerotia from stock cultures. Plates were incubated at room temperature for 60 to 70 days. At the end of this growth period, about 40% of the agar surface was covered with a thick, black microsclerotial mat; the remainder of the medium was fairly uniformly colonized with a less dense type of microsclerotial growth. Inoculum furnishing what is referred to in this paper as the "standard dosage" or fractions thereof, was obtained by blending the fungus–agar contents from one Petri plate with 400 ml of tap water for 90 seconds in a Waring Blendor. Twenty milliliters of this suspension were thoroughly mixed with the soil in each of the pots to be inoculated. Similarly comminuted sterile agar was added to the soil of those pots which were not inoculated with the fungus.

The concentration of *V. albo-atrum* used in the various tests consisted of "standard" and 1/4, 1/12, and 1/36 "standard".

Pratylenchus penetrans used in these tests was originally obtained from the roots of vegetables in southwestern Ontario and maintained in the greenhouse on the roots of vetch (*Vicia villosa* Roth) for approximately two years. In the preparation of nematode inoculum, infested vetch roots were washed thoroughly, chopped, and placed on a nylon screen in a glass funnel, filled with a solution containing both a bactericide and a fungicide. The procedure, exactly as described by Mountain and Patrick (7), yielded active, infective nematodes, free from culturable bacteria and fungi. With one exception, approximately 2600 *P. penetrans* were added to each pot requiring such inoculum.

Five-inch clay pots, with the drain holes tightly stoppered, were sterilized and filled with a steam-sterilized compost soil consisting of approximately two-thirds Fox sandy loam and one-third muck. These pots were then placed in a plastic tray (four per tray) and the remaining space in the tray was filled with sterile sand as described previously (7). In each test, four treatments were used: the nematode alone, the fungus alone, the nematode plus the fungus, and a non-inoculated control. Each treatment consisted of 10 single-pot replications.

Seeds of eggplant, variety Imperial Black Beauty, were germinated in a growth room in sterile quartz sand watered with a nutrient solution as required. The seedlings were transplanted, four to each test pot, when they were 6 to 8 cm tall, at which time the first true leaf was approximately 15 mm in diameter.

The tests were carried out in a growth room in which the soil temperature was maintained at 75° F; the air temperature was held at 72° F during illumination and 65° F during darkness. The plants received approximately 1200 ft-c of artificial illumination during a 16-hour day. The pots were watered periodically with "starter" solutions and other nutrients to maintain good vegetative growth. Growth of the eggplants was very satisfactory under the imposed conditions.

The numbers of wilted plants were counted on alternate days in all treatments for 40 days. Symptoms of *Verticillium* wilt were clear-cut, the earliest being flaccidity of the leaf margins. Simultaneously, flaccid areas became chlorotic. Wilted leaves, or portions thereof, became progressively yellowed and eventually died. The fungus could usually be easily isolated from the bases of the petiole of affected leaves.

After 60 days, the plants were removed from the soil, the roots carefully washed, and the weight of both roots and tops obtained immediately. The nematodes were then extracted from the roots by a modification of Young's method (16) as follows: washed roots were placed in pint Mason jars and covered by 250 ml of a solution containing 2 p.p.m. ethoxyethyl mercury chloride and 500 p.p.m. dihydrostreptomycin sulphate. Distilled water was added as required to replace evaporation losses. The jars were then stored at 65° F and aerated individually and continuously for a period of 4 weeks. The numbers of *P. penetrans* which emerged from the roots were then counted.

Results

The results of the experiments outlined above are depicted graphically in Fig. 1 and summarized in Table I. From Fig. 1 it is apparent that the nematode appreciably influenced the incidence of wilt at all levels of fungus inoculum except at the "standard dosage". In one experiment (1/36 standard dosage), where a higher nematode population was used, there was evidence of a direct relation between the number of nematodes and the incidence of wilt. Table I indicates that the fungus also appreciably affects the nematode. At the medium (1/12) and lower (1/36) dosage rates of fungus inoculum, the root populations of *P. penetrans* were significantly higher in the presence than in the absence of *V. albo-atrum*. However, as the fungus inoculum was increased this effect disappeared.

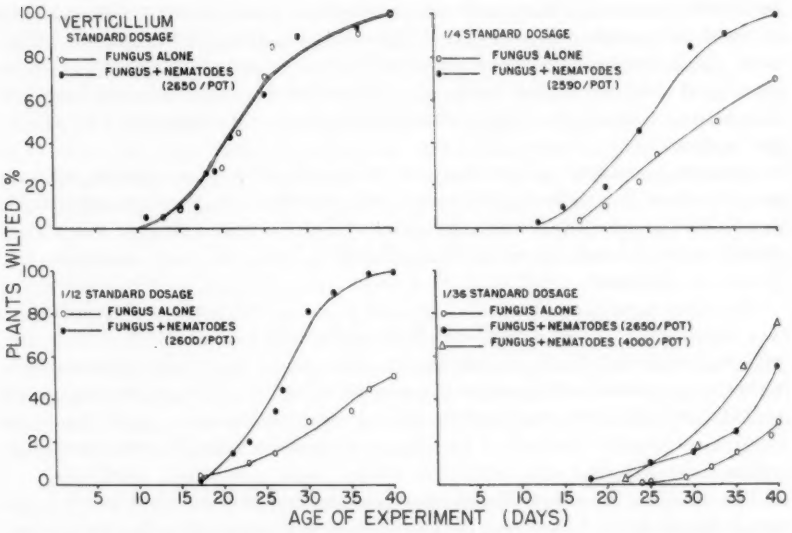

FIG. 1. The incidence of wilt in eggplant at four inoculum levels of *V. albo-atrum* in the presence and absence of *P. penetrans*.

On the basis of generally accepted criteria, *P. penetrans* was not pathogenic to eggplant. Even with dosages as high as 4000 nematodes per pot, there was no significant reduction in the growth of the plant. No effect on root growth could be discerned and, by visual examination, nematode-infested roots could not be distinguished from those of the controls. Microscopic examination of infested roots showed rather indistinct, tan-colored areas, but necrosis and root tip abnormalities could not be detected.

The results indicate a synergistic interaction between the two parasites for, at certain levels, the incidence of wilt was increased by the presence of the nematode and conversely the number of nematodes within the root by the presence of the fungus.

TABLE I

The effect of *V. albo-atrum* on the population of *P. penetrans* in eggplant roots

Amount of *Verticillium* added	Soil inoculation	*Pratylenchus penetrans*	
		No. added per pot	No. recovered per pot
Standard dosage	N[a]	2650	738
	NV[b]		786
1/4 standard	N	2590	1003
	NV		1496
1/12 standard	N	2600	921
	NV		2010**
1/36 standard	N	2650	744
	NV		1410**
1/36 standard	N	4000	2908
	NV		3680**

[a]*P. penetrans* alone.
[b]*P. penetrans* + *V. albo-atrum*.
**Significantly greater than in corresponding N series at 1%.

Discussion

The possible existence in the field of a similar synergistic interaction of *Verticillium albo-atrum* and *Pratylenchus penetrans* is most intriguing but must be discussed with caution at this stage. In the first place, field investigations of this phenomenon have not yet been carried out. Then too, the relationship described in this paper involves the study of only two of the many microorganisms which must be present in the soil environment of eggplant roots. Also, it would appear that an interaction may exist only if the fungus and nematode occur together at certain population levels. Field studies of nematode populations in relation to the occurrence of wilt and comparative tests in the growth room of naturally infested field soil and artificially inoculated test soils should establish the importance of nematodes in the etiology of *Verticillium* wilt under natural conditions.

If such an interaction does occur to an appreciable extent in the field, it would help to explain the epidemiology of *Verticillium* hadromycosis in Ontario. Previously, the senior author (6) found that an outbreak of *Verticillium* wilt in the Niagara peninsula in Ontario was contingent upon a combination of high soil moisture and low-to-medium soil temperatures during the early part of the growing season. Unpublished research by the second author indicates that such conditions are favorable for the activity of *P. penetrans*.

The observation that, at certain inoculum levels of *V. albo-atrum*, significantly higher root populations of nematodes occur in the presence of the fungus is intriguing and warrants detailed study. Since the proportion of juvenile to adult nematodes was usually greater when the fungus was present, it is possible that the fungus rendered the root substrate more suitable for nematode reproduction. Since Pegg and Selman (9) showed that *V. albo-atrum*

produces indole-3-acetic acid in culture and in tomato stems, undoubtedly invaded tissues of eggplant root are also affected by this fungus. As the effect on the nematode disappeared at high levels of fungus inoculum, *V. albo-atrum* may eventually compete with *P. penetrans* for available nutrients, although several other possibilities could be postulated at this stage. In any event, the observation points up rather sharply the danger of nematologists ignoring possible effects of root parasitic microorganisms when studying and interpreting population dynamics of plant parasitic nematodes.

There are several possible mechanisms by which the nematode effects the increase in the incidence of wilt. There may be a direct mechanical effect in which the nematode provides the avenue of entrance into the root and perhaps disrupts the cellular structure of the cortical tissue, thereby facilitating the entrance of the fungus into the stele. Or, the effect may be chemical, and enzymes secreted by the nematode (7) may provide a more suitable substrate for the nutrition of the fungus. Since Selman and Buckley (12) recently found that root damage to tomato seedlings by transplanting or by deliberate scalpel wounds resulted in greatly accelerated systemic invasion by the fungus, it is possible that the nematode may exert its influence through mechanical means. Histological studies should show the type of colonization of eggplant roots by *P. penetrans* and indicate the operative mechanism.

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ONTOGENETIC TRENDS IN CONIFER CAMBIUM WITH RESPECT TO FREQUENCY OF ANTICLINAL DIVISION AND CELL LENGTH[1]

M. W. BANNAN

Abstract

Through the growth of the first few years, when the perimeter of the stem is fast expanding, the multiplicative pseudotransverse divisions in fusiform cambial cells occur in relatively rapid sequence, the ratio of survival of newly formed cambial cells is high, and continuous increase in mean cell length ensues. Accommodation of the cambium to circumferential expansion is thus facilitated. With increasing age and diminishing rate of perimeter growth the frequency of pseudotransverse division declines, the survival rate of new-formed cambial initials drops, and increase in cell length slackens. This gradual flattening off may continue through to the final growth, but more often fluctuations in rate of anticlinal division and cell length arise in the middle to late growth, these not always exhibiting close interrelationship. Sometimes high rates of pseudotransverse division occur in the late growth which are accompanied by only moderate reductions in cell length.

Introduction

From observations on *Pinus sylvestris*, Sanio (26) concluded that tracheid length increased outward from the pith, through a number of annual rings, until a definite size was reached which remained constant through the following rings. Later, Bailey and Shepard (4) stated that, after an early increase in length, fluctuations occurred in the outer rings. Numerous workers (1, 3, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, and 28) have since confirmed the continuous increase in cell length in the innermost rings but various trends have been noted in the later growth.

Diameter growth is accompanied by circumferential expansion of the cambium, which entails addition to the number of cambial initials. This is accomplished by pseudotransverse division (2, 6, 21 29) by means of which a semitransverse wall is laid down, usually near the center of the dividing cambial cell. The two daughter cambial cells so formed subsequently elongate by tip growth to full size when the sequence of multiplicative division and cell elongation may be repeated. In the present paper, data will be presented on the changes in frequency of pseudotransverse division and cell length that accompany diameter increase.

Material and Methods

The frequency of pseudotransverse division at various stages in the development of the stem was determined by the method mentioned in an earlier paper (8). Serial tangential sections were cut of the secondary xylem from blocks taken at specific distances from the pith. By tedious microscopic examination the locations of pseudotransverse divisions were noted in as many as 50 random lineal files of elements and the information recorded. Some typical

[1]Manuscript received May 24, 1960.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Assistance was provided by a grant in aid of research furnished by the University of Toronto.

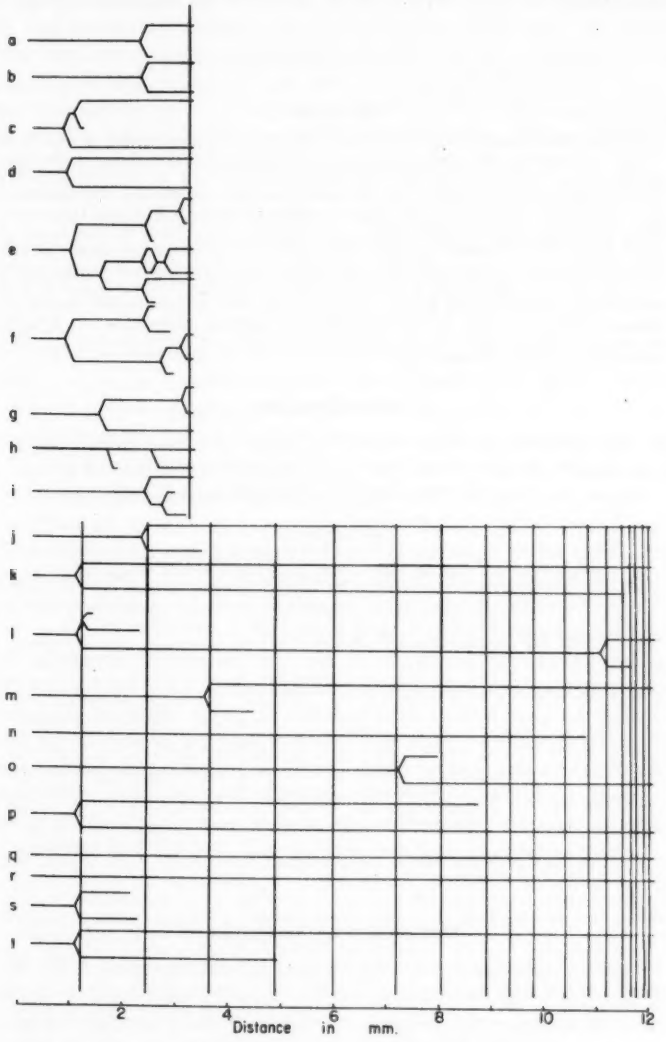

FIG. 1. Diagrams showing the relative time and frequency of pseudotransverse divisions involved in the multiplication of fusiform initials in a stem of *Thuja occidentalis*. Horizontal lines represent individual case histories. Vertical lines denote boundaries of annual rings. Series *a–i* are from the inner growth, 1 cm from the pith, and series *j–t* are from the outer growth, 12 cm from the pith.

case histories are shown in Fig. 1. Thus series *a* depicts an instance where one pseudotransverse division occurred during the production of the annual ring, the division taking place toward the end of the growth for the year. One of the daughter initials survived to continue producing xylem elements, whereas the other was soon lost from the cambium. In series *b* a single pseudotransverse division was followed by survival of both daughter initials (see Fig. 2, *a–c*). More frequent divisions occurred in series *e*. Here a succession of three pseudotransverse divisions occurred in the growth of the year. The diamond-shaped area which appears in the diagram denotes a temporary division. The cross wall persisted through the production of 135 μ of xylem before disappearance (Fig. 2, *d–f*). In this case the pseudotransverse division evidently took place in a xylem mother cell rather than in the fusiform initial. Yet another circumstance is shown in series *h* where there were two successive divisions to produce segments off the side. The first and smaller segment soon failed (Fig. 2, *h*, *i*), whereas the second and larger segment persisted to become

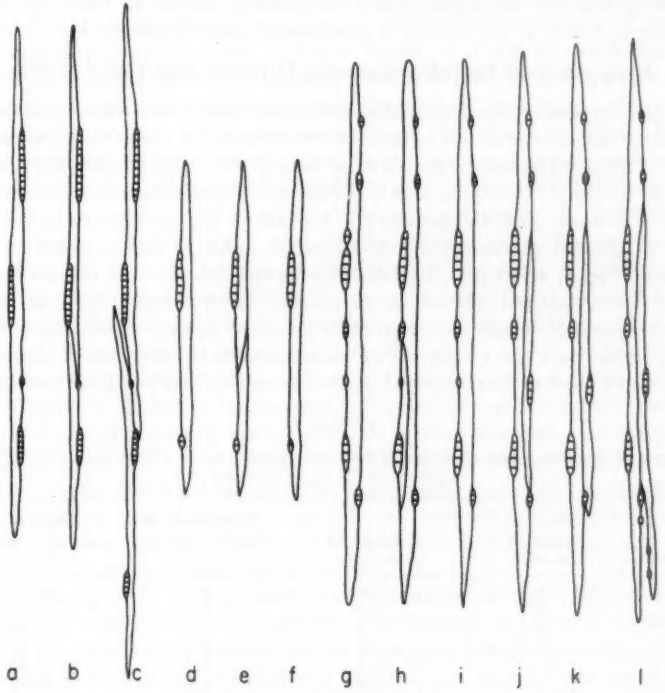

FIG. 2. Drawings from serial tangential sections illustrating the sequence of changes associated with anticlinal division. Series *a–c* shows a pseudotransverse division and survival of both new-formed daughter initials (series *b*, Fig. 1). Series *d–f* shows a temporary pseudotransverse division, i.e. a division of a xylem mother cell (see series *e*, Fig. 1). Series *g–l* shows two successive divisions to cut segments from the side of a fusiform initial (series *h*, Fig. 1).

a functioning fusiform initial (Fig. 2, *j–l*). Such divisions, namely pseudotransverse division of xylem mother cells or the cutting off of segments from the side of cambial cells, constitute only a small proportion of anticlinal divisions in the cambium (9).

The frequency of pseudotransverse division was calculated as follows. In Fig. 1, series *a*, where one division occurred during the production of 3.0 mm of xylem, the frequency was rated as 3.3 divisions per cm of radial increment. In series *e* where three successive divisions took place during the development of the annual ring, the rate was calculated as 10 divisions per cm of xylem. An average value was arrived at by using the formula

$$r = (s \times 10)/(n \times t)$$

where r = the frequency of pseudotransverse division per cm of xylem production, s = the number of *successive* pseudotransverse divisions per series, n = the number of series, and t = the thickness of xylem sectioned as measured in millimeters. It should be noted that the transverse division of obviously collapsing cells and the segmentation of fusiform initials to form ray initials were not included in the counts of pseudotransverse divisions.

Frequency of Pseudotransverse Division and Cell Length

For the purpose of illustrating the ontogenetic trends in frequency of pseudotransverse division and cell length, reference will be made to a 60-year-old tree of *Thuja occidentalis*, the stem of which was approximately 2.3 dm in diameter at the 3-ft level. In this stem the annual increments decreased from widths of 2–4 mm near the pith to 0.2–1.2 mm in the periphery. In the early stages of growth pseudotransverse divisions followed one another in rapid sequence (Fig. 1, series *a–l*), but with increasing age the rate of pseudotransverse division declined relative to the production of xylem (Fig. 1, series *j–t*). Data are given in Table I for two radii intersecting at a right angle. Here it will be noted that the frequency of pseudotransverse division dropped from 5.1 per cm of xylem increment at 1 cm outwards from the pith to values of

TABLE I

Frequency of pseudotransverse divisions and cell length at ptr. division in stem of *Thuja occidentalis* 23 cm in diameter

Radius	Distance from pith in cm	Ring width in mm	Frequency of divisions per cm of xylem	Length of cells at ptr. division in mm
A	1	3.0	5.1	1.64
	4	3.1	3.4	2.42
	6	4.3	1.3	2.50
	9	1.2	2.3	2.53
	11	0.8	2.8	2.40
		0.2	4.0	2.52
B	3	2.1	3.7	2.30
	6	2.6	0.8	2.80
	12	1.4	2.0	2.85
	13	1.2	1.0	2.87
		0.6	0.2	2.91
		0.2	1.5	2.81

0.2–2.8 in the peripheral growth. The increase in the outermost growth in radius A was due to the development of a groove, an irregularity that is characteristically accompanied by a rise in the frequency of pseudotransverse division (8).

The high frequency of pseudotransverse division in the early growth was accompanied by comparative shortness of the cells (see Table I). A slowing up of the frequency of multiplicative division in the following growth was paralleled by an increase in cell length. These changes took place early, during the development of the first few centimeters of xylem. Changes were also noted in the ratio of survival of fusiform initials newly formed in pseudotransverse division. During the deposition of the xylem 1–4 cm outwards from the pith, the net gain in fusiform initials was 30–50% of the potential. With increasing cambial age the ratio of loss of initials relative to the frequency of multiplicative division rose so that a status quo was approximated. Despite the continuation of a relatively high frequency of pseudotransverse division, and hence the production of many new initials, loss from the cambium was so great that little or no gain in number of initials accrued in the later stages of growth. Sometimes there was a net loss. In Fig. 1, series *j–t*, it will be noted that after 12 multiplicative divisions the original 11 fusiform initials were reduced to 10.

Differences were also noted between the earliest and later growth with respect to the time at which pseudotransverse divisions took place. During the development of the earliest wood multiplicative divisions occurred at different times during the growing season (Fig. 1, series *a–i*). This, of course, facilitates rapid and continuous circumferential expansion. In later growth, when perimeter extension from year to year steadily decreases, pseudotransverse divisions tend to become more definitely an aestival phenomenon, taking place near or at the end of the growing season (Fig. 1, series *j–t*).

Trends similar to those described for *Thuja* were observed in trees of *Cedrus*, *Picea*, and *Pseudotsuga*. It seems clear that during the growth of the first few years, when the perimeter of the stem is increasing rapidly, the multiplicative pseudotransverse divisions in the cambium occur in rapid sequence, the ratio of survival of newly formed fusiform initials is relatively high, and continuous increase in mean cell length follows. All of these phenomena facilitate accommodation of the cambium to circumferential expansion. With increasing age and diminishing rate of perimeter growth the frequency of pseudotransverse division declines, the survival ratio of new-formed fusiform initials drops sharply, and increase in cell length slackens.

In the later growth of large stems, various situations obtain. Sometimes the trends noted in the early growth continue. For instance, in a stem of *Pseudotsuga taxifolia* 2.2 meters in diameter, the frequency of pseudotransverse division soon reached a very low level, being 0.6, 0.7, 0.8, and 0.1 per cm of radial accretion at distances of 2, 7, 10, and 10.5 dm from the pith. Mean cell length at pseudotransverse division increased progressively from 4.75 mm at 2 dm from the pith to 5.35, 5.85, and 5.89 mm at the successive distances indicated. The cambium here conformed to the commonly held concept of the meristem as static.

The more prevalent condition in the middle-to-late growth of old stems is apparently that characterized by fluctuation of varying amplitude in cell length. Harlow (16) observed this phenomenon in *Thuja*, Lee and Smith (23) in *Pseudotsuga*, Liang (24) in *Larix*, Bisset, Dadswell, and Wardrop (13) in *Pinus*, and Bailey and Faull (3) in *Sequoia*. In several boles of *Sequoia sempervirens* and *S. gigantea* ranging from 1.3 to 6 meters in diameter the frequency of pseudotransverse division in the outermost rings varied from 1.1 to 5.0 per cm of radial increment. In the stems with the higher frequencies of multiplicative division the cambium was in a state of flux with quickly changing cell patterns. Despite the relatively high frequency of multiplicative division the cambial cells were long, mean length at pseudotransverse division ranging from 4.09 to 7.53 mm in different trees.

Sometimes the fluctuations in frequency of multiplicative division and cell length in the middle and late growth of stems are due to special circumstances such as the development of fluting, grooves, or similar departures from the normal rounded contour (8). Growth intensity, as reflected in ring width, also has some influence on cell length but not on the frequency of multiplicative division (10.) Much of the fluctuation, however, cannot be related to the above variants and apparently is due to causes as yet undetermined.

Discussion

In their search for the mechanism involved in the expansion of cell size outward from the pith, certain authors have emphasized the relationship between cell length and distance from the pith. Anderson (1) concluded that this relationship could be expressed in mathematical terms. Hejnowicz and Hejnowicz (19), working with *Populus*, made similar observations and deduced that the important factor in increase in cell length was not so much actual distance as the number of cambial generations involved, i.e. the number of times that the cambial cells divided. This is an interesting hypothesis but difficulty is encountered in trying to prove or disprove it because of the organization of the cambium. The generative zone consists of the uniseriate tier of initiating cells and derived mother cells on either side. In actively growing trees the xylem mother cells make up a zone several cells wide and much redivision occurs before maturation. Thus for every periclinal division of the fusiform initials several xylem cells result (7). The relative cambial age (Hejnowicz and Hejnowicz), or number of cambial generations, must obviously relate to the number of times that the permanently meristematic cells (fusiform initials) divide. This value is not revealed in a count of wood cells, and estimates are apt to be greatly in error because the production of xylem cells per division of fusiform initials varies not only with the rate of growth but also fluctuates among trees of similar growth rates.

Through the early growth, when the cambium is young, the frequency of pseudotransverse division tends to drop and cell length to increase in the expected inverse relationship. With advancing age, fluctuations often develop, the variations in cell length and frequency of multiplicative division at times

showing little or no relationship to one another. Sometimes the rate of pseudotransverse division in the peripheral growth of large stems is surprisingly high, but, even in those cases where the frequency of division is much above average, cell length is only slightly reduced. At least in the peripheral growth the frequency of pseudotransverse division seems to be related not to circumferential expansion or number of periclinal divisions in the fusiform initials but rather to linear radial distance (10). A mechanism apparently exists whereby provision is made for continuous cambial renewal without detrimental effects on cell length.

The variability that exists in different parts of the tree creates a problem for the forest-tree breeder interested in cell size. For practical reasons measurements of cell size are usually taken from the inner wood and the assumption is made that the data so obtained are valid for comparative purposes. Since cell length tends to increase rapidly in the early growth it is obvious that care must be exercised in the selection of the particular wood used for sampling.

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AQUATIC HYPHOMYCETES FROM CANADA[1]

C. T. INGOLD

Abstract

Fifteen species of aquatic Hyphomycetes are described from Canada. Further, some spore types are illustrated which do not seem to belong to known species.

For many years I have been interested in the aquatic Hyphomycetes which grow on submerged decaying leaves of broad-leaved trees and shrubs in well-aerated waters, especially streams. The literature on the subject has recently been fairly completely listed (Ingold 1959).

Whilst visiting Canada in connection with the International Botanical Conference I had opportunities, unfortunately all too few, of observing these fungi. Since it does not appear that there are any previous records from Canada, my scattered observations may be of some interest, and may encourage others to make a real study of these organisms in that country.

The fungi occur particularly on the petioles and larger veins of the submerged decaying leaves. The spores are liberated into the water.

Few species have round or oval spores. In a number they are very long and habitually sigmoid with the curvature lying in more than one plane. In most, however, the spore is a branched structure and this most often consists of four long, divergent arms. This tetraradiate type of spore develops in such very different ways in different genera that clearly parallel evolution is involved, indicating that it possesses some special value in the aquatic environment. Probably this value lies in a spore of this form acting as a miniature anchor, since arrest on a suitable substratum may be a real problem in the dispersal story of these fungi living, as they so often do, in rapidly flowing water.

Although slightly heavier than water, the spores are readily trapped in foam or scum which may accumulate behind a barrier of twigs or stones or at the foot of a small waterfall in a stream. If this foam or scum is skimmed off with a spoon and examined, it is usually found to be full of these spores, and so distinctive are most of them that individual species are identifiable on the structure of the spores alone.

During the pre-Congress excursion to the Gaspé Peninsula (11–18 August), collections of scum were made from three small streams, and, on a mid-Congress excursion to Lake Ouimet near Mont Tremblant (22 August), scum and decaying leaves (mainly *Acer* spp.) were obtained from little torrents careering down through mixed forest to the lake. In one of these collections the scum appeared to consist entirely of the spores of aquatic Hyphomycetes.

[1]Manuscript received April 7, 1960.
Contribution from Birkbeck College, University of London, Malet Street, London, W.C. 1, England.

TABLE I

Species	Locality			
	Gaspé Peninsula			Laurentian Playground, Lake Ouimet (near Mont Tremblant)
	Anse au Griffon	Percé	Vauquelin	
Alatospora acuminata Ing.	+	+	+	+
Anguillospora longissima (de Wild.) Ing.	+	+	+	+
A. crassa Ing.	+	+	+	+
Ankistrocladium fuscum Perrott				+
Articulospora tetracladia Ing.			+	+
Clavariopsis aquatica de Wild.	+	+	+	+
Culicidospora aquatica Petersen	+	+		+
Dendrospora erecta Ing.				+
Flagellospora curvula Ing.	+			+
Heliscus longibrachiatus Ing.			+	
Lemonniera aquatica de Wild.	+	+	+	
L. brachycladia Ing.	+		+	
Nectria lugdunensis (Sacc.) Webster (conidial stage)	+	+	+	+
Tetracladium marchalianum de Wild.	+	+		
Tricladium angulatum Ing.			+	+?

Records of species identified are shown in Table I.

Although many of the spores in the samples could readily be identified, there were others that clearly belonged to undescribed species.

Spores found in Gaspé and also at Ouimet (Fig. 1, A) certainly belong to *Culicidospora*, a genus recently described (Petersen 1960) from near New York, and probably to the type species *C. aquatica*.

Another type of spore from Anse au Griffon in Gaspé is composed of a main axis and three or four laterals, one of which occasionally itself branches (Fig. 1, C). All these ramuli are monilioid with bead-like individual cells.

A few spores were seen in the Percé collection which clearly belong to an undescribed species of *Dendrospora*. The axes are, however, shorter and stouter than in the only described species, *D. erecta*, and the number of laterals above the basal whorl is very small (Fig. 1, B). These spores are of interest because they seem to suggest a link between *Dendrospora* and *Triscelophorus*.

The spore-containing scum from Ouimet was dominated by *Articulospora tetracladia*. It also contained many of the characteristic spores of *Dendrospora erecta*. Further, there was present in some quantity a spore type probably referable to *Triscelophorus*. These spores are much larger than those of *T. monosporus*. Each has three, or sometimes only two, branches arising near the base of the main axis. The striking feature is the tendency, not always realized, of these laterals to branch again and reproduce, to a greater or lesser degree, the branching pattern of the main axis. In *Dendrospora erecta* one or more of the laterals of the lowest whorl themselves branch near the point of origin. Thus this undescribed *Triscelophorus* from Ouimet shows some approach towards *Dendrospora erecta*, just as the undescribed *Dendrospora* from Percé approaches *Triscelophorus monosporus*.

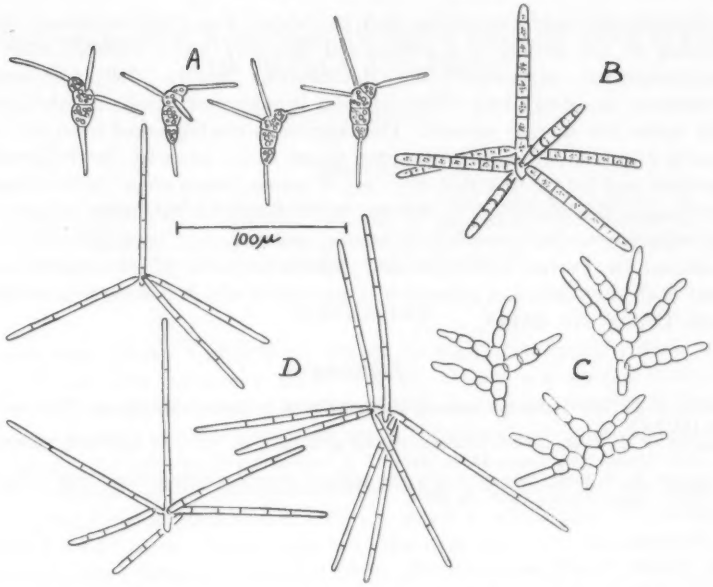

FIG. 1. (A) *Culicidospora aquatica*: left, two spores from Anse au Griffon; right, two from Ouimet. (B) *Dendrospora* sp. from Anse au Griffon. (C) Spores of an unknown fungus from Anse au Griffon; (D) *Triscelophorus* sp. from Ouimet.

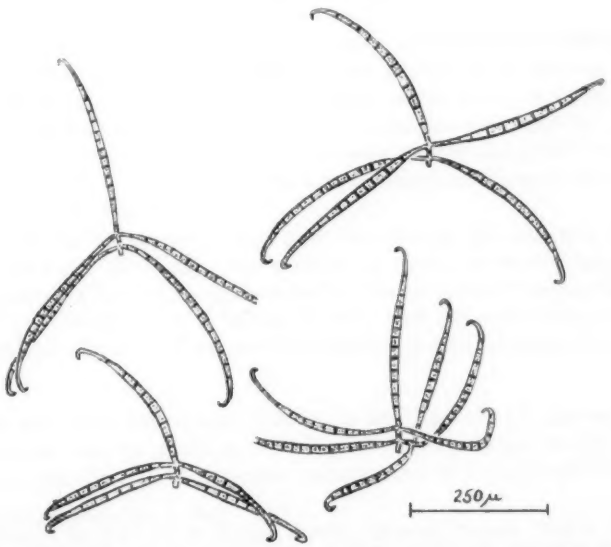

FIG. 2. *Ankistrocladium fuscum*: spores from Ouimet. Drawn from Herb. DAOM 64143.

Perhaps the most interesting find at Ouimet was *Ankistrocladium fuscum* growing on the petiole of a submerged decaying leaf (probably *Acer* sp.). This fungus has very recently been described by Perrott (1960), who found it developing on an oak twig collected from Windermere, England, and kept in cold water for several months. This species is distinguished from the other aquatic Hyphomycetes with branched spores that I know by the thick walls of the spore and by the fact that they are of a rust-brown color. In this remarkable fungus the spores are enormous, individually visible under a hand lens, and each arm of the spore is stiff, brittle, and ends in a hook (Fig. 2). I have examined Dr. Perrott's material and there is no doubt of its complete agreement with the Canadian collection, a sample of which has been deposited in Herb. DAOM No. 64143.

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PHOTOPERIODIC RESPONSE IN SEEDLINGS OF FIVE SPECIES OF BETULA AND PINUS[1]

O. VAARTAJA

Abstract

The existence of photoperiodic response was experimentally established in *Betula lenta*, *B. mandshurica* var. *japonica*, *B. glandulosa*, *Pinus lambertiana*, and *P. monticola*. The responses of seedlings from different latitudes and altitudes were in accordance with the theory of photoperiodic ecotypes.

Introduction

Long days hasten and prolong growth of many tree species, while short days induce early dormancy (1, 2, 3, 4, 5). Only a few species have been found that are not, or are only slightly, affected by daylength (3, 5). The response is small in most trees from warm climates in low latitudes (ca. 30°), but large in all from high latitudes (1, 2, 3, 4).

The existence of the photoperiodic response is explored in this paper for sugar pine (*Pinus lambertiana* Dougl.), western white pine (*P. monticola* Dougl.), sweet birch (*Betula lenta* L.), Japanese birch (*B. mandshurica* var. *japonica* (Mig.) Rehd.), and dwarf birch (*B. glandulosa* Mich.). White birch (*B. papyrifera* Marsh.), which has already been tested (2, 3, 6), is included as a control.

1. *Betula*

Methods

To help the birch seedlings over the usual establishment difficulties (3), the germinating seeds were planted in jars containing a mixture of forest humus, sphagnum moss, and sterilized sand (ratio 1:4:5), and placed for a month under continuous illumination. Then the jars were kept for 4 months under the extremely long (ELD) and extremely short day (ESD) treatments in growth chambers. The treatments were arranged in the same way as in an earlier study (3) (Fig. 1).

The illumination intensity was 2000 ft-c during the day and 1000 ft-c during the night. The light was provided by cool, white fluorescent tubes supplemented (5%) by incandescent bulbs. The air temperature varied usually from 15° C at night to 30° C during the day. The jars were half-immersed in water tanks at about 19° C to provide a suitably low temperature for the roots.

Results

At the end of the experiment, one to five seedlings of each species survived and appeared healthy under each treatment. Their average growth is shown in Table I and Fig. 2. The responses were correlated with latitude of the seed

[1]Manuscript received May 5, 1960.
Contribution No. 639, Forest Biology Division, Research Branch, Canada Agriculture, Ottawa, and No. 53, Canada Agriculture Research Station, Saskatoon, Saskatchewan, Canada.

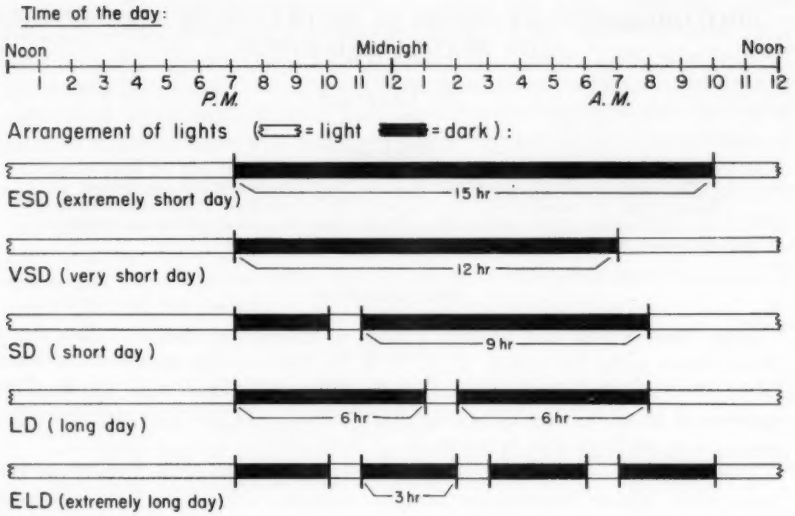


FIG. 1. Arrangement of various daylength effects in five treatments giving 1-hour light periods during the dark period in three of the treatments.

source, that is, the farther north the origin, the more was the growth suppressed by ESD. This is in accordance with the earlier results with a large number of species (3, 4).

All the birch species responded to the treatments, but in *B. japonica* from a relatively warm climate the response was obvious only in height growth (Fig. 2). The experiment was repeated with this species, and the results were similar. While the differences in weight were negligible, the average height of seedlings grown under short days was only 56% of that under long days. Obviously, a daylength that inhibits elongation does not necessarily inhibit the growth of cambium or roots. This has also been found in *B. papyrifera* (3).

B. glandulosa, a bog shrub in nature, was able to grow almost as fast as *B. lenta* and faster than the other species; perhaps it was favored by the

TABLE I

Growth of seedlings of *Betula* spp. after 4 months under long and short day treatments (ESD and ELD, Fig. 1)

Species and latitude	Stem length, cm		Dry weight of top, mg		Total dry weight, mg		No. of leaves	
	ESD	ELD	ESD	ELD	ESD	ELD	ESD	ELD
B. japonica, ca. 40°	5	8	120	120	140	140	2.3	4.2
B. lenta, ca. 42°	12	18	280	500	320	570	10.0	10.3
B. papyrifera, 47°	5	10	110	340	140	400	3.6	5.3
B. glandulosa, 54°	4	16	10	490	10	530	3.0	11.0

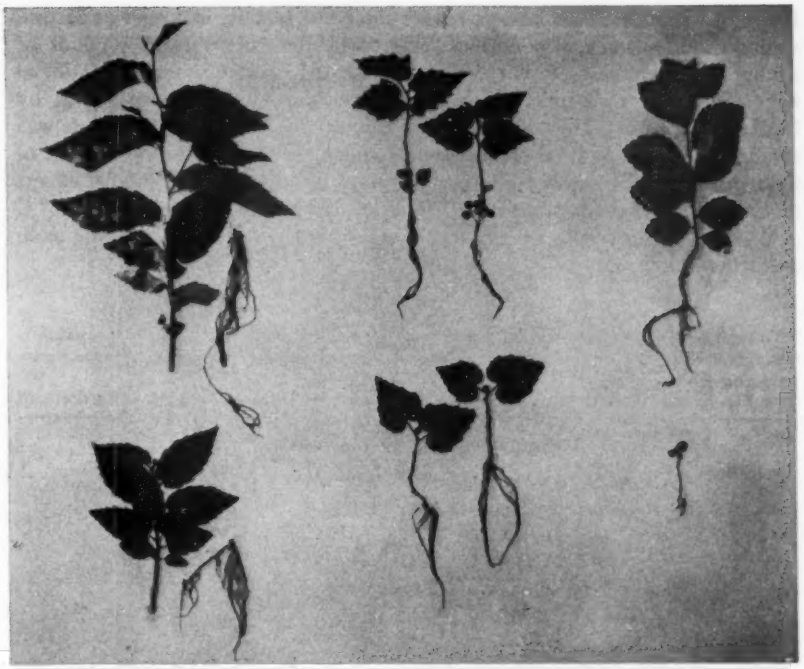


FIG. 2. Growth of *Betula lenta* (left), *B. mandshurica* var. *japonica* (middle), and *B. glandulosa* (right) seedlings under long days (upper row) and short days (lower row). (Treatments designated by ELD and ESD in Fig. 1.)

rather acid soil used. Under ESD the tips of the seedlings appeared rosetted due to the short internodes typical (3) of short day response. The leaves of *B. glandulosa* were abnormally small under ESD and abnormally large under ELD (Fig. 2).

2. *Pinus lambertiana*

Methods

Seed was obtained from Oregon and California from (*a*) 43° N., elev. 5000 ft; (*b*) 39°, 5500 ft; (*c*) 37°, 6300 ft; (*d*) 38°, 500 ft. The seeds were stratified and germinated in sphagnum moss under continuous illumination. The young seedlings were transplanted in pots containing a mixture of forest humus, sphagnum moss, and sterilized sand (ratio 1:5:4). The pots were arranged in pairs, the sources (*a*) and (*b*) forming one set of pairs, and (*c*) and (*d*) another set. This was done to provide the most accurate comparison between the northern (Ore.) and southern (Calif.) sources from approximately the same elevation, on the one hand, and between very high and very low elevation (6300 and 500 ft) from approximately the same latitude, on the other hand.

Three pots of each seed source were kept for 4 months in five greenhouse compartments. The experiment was started during very short days in winter.

Black curtains were used later to reduce the hours of daily exposure to natural sunlight. Each compartment received approximately the same amount of sunlight with maximum intensity about 10,000 ft-c; they also received artificial light (500 ft-c) from cool, white fluorescent tubes supplemented (10%) by incandescent bulbs. The timing of these lights in different compartments was arranged as shown in Fig. 1. This arrangement provided the same amount of light in four of the compartments, but photoperiodically different (3) effects in each of the five compartments. The air temperature varied from 15° C at night to 30° C during the day. The method was essentially the same as in an earlier study (3).

TABLE II

Growth of *P. lambertiana* seedlings after 4 months under different daylength treatments

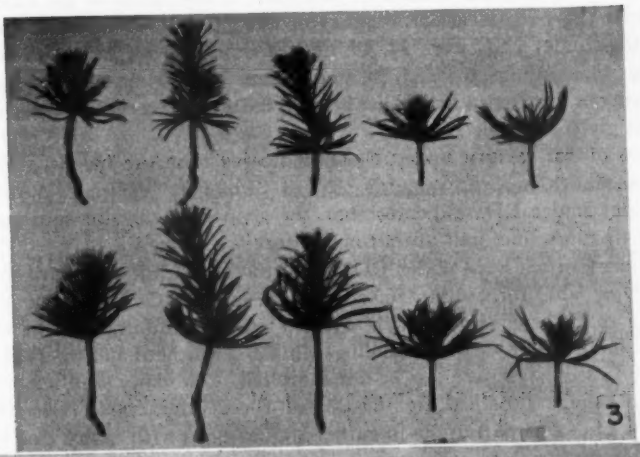
Treatment (see Fig. 1)	Stem length	Dry weight of top	Total dry weight	Tips dormant
	I. Averages from all four seed sources (cm mg)			
ESD	7	260	500	Yes
VSD	7	320	550	Mostly yes
SD	9	480	800	Mostly yes
LD	12	450	700	No
VLD	11	400	600	No
	II. Ratios source *a*/source *b* (= Oregon/California)			
ESD	.8	.9	.8	—
VSD	.8	.8	.9	—
SD	.8	.7	.7	—
LD	.7	.6	.9	—
VLD	1.0	.9	1.1	—
	III. Ratios source *c*/source *d* (= elev. 6300 ft/elev. 500 ft)			
ESD	1.2	1.2	1.3	—
VSD	.7	.7	.7	—
SD	.8	.9	1.0	—
SD	1.2	.9	1.0	—
VLD	1.0	1.1	1.0	—

Results

Table II and Fig. 3 show that *P. lambertiana* seedlings responded photoperiodically. Under ESD and VSD the seedlings became dormant earlier than under the other treatments and grew very little. The most obvious inhibitory effects were in height growth and weight of top. Height growth was inhibited in some of the Oregon seedlings even by LD, and in some of the high-altitude seedlings by SD. VSD and ESD inhibited the height growth of all seedlings.

FIG. 3. Growth of high-altitude (upper row) and low-altitude (lower row) seedlings of *Pinus lambertiana* under different daylengths (decreasing daylengths from left to right, arranged as in Fig. 1).

FIG. 4. Growth of northern (upper row) and southern (lower row) seedlings of *Pinus monticola* under three daylength treatments (from left to right: LD, SD, VSD; see Fig. 1).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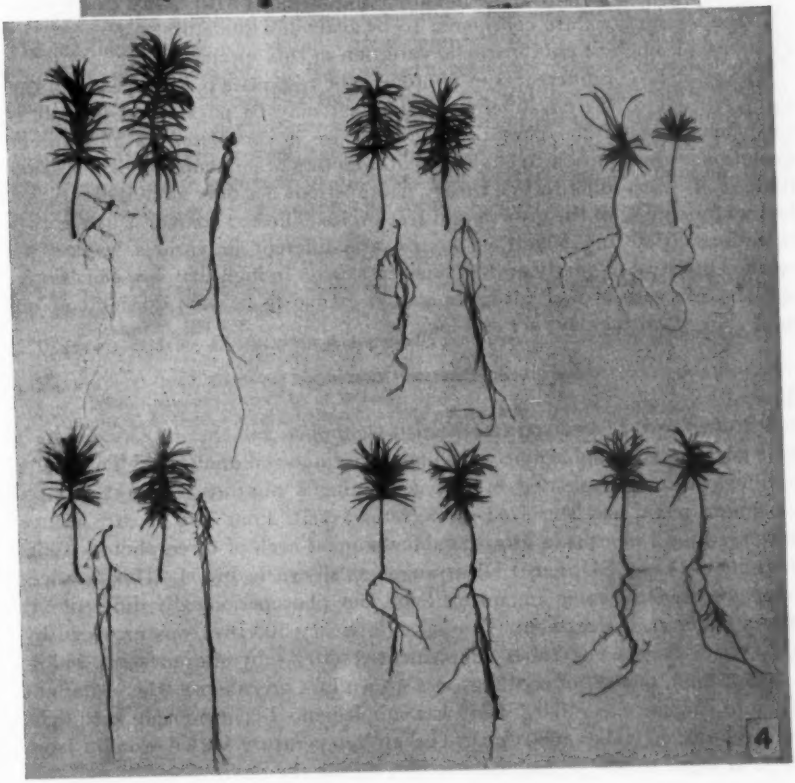
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The following example illustrates how each of the ratios in the table were calculated: the heights (stem lengths) of Oregon (*a*) seedlings in the three replications under ESD were 44, 49, and 54 mm. The heights of their pairs, California (*b*) seedlings, were 53, 56, and 67 mm. By dividing the corresponding values one obtains the ratios 0.83, 0.88, and 0.81. The average of these is 0.84, or 0.8, as given in Table II.

A ratio of 1.0 (length under ELD) indicates that the height growth of Oregon seedlings was the same as that of California seedlings. A ratio 0.6 (top weight under LD) indicates that the growth of Oregon seedlings was only a little more than half of that of California seedlings.

The lowest ratios were under LD or SD for Ore./Calif. comparisons, and under SD or VSD for 6300-ft/500-ft comparisons. This can be explained by assuming that these photoperiods exerted strong inhibitory effect on the northern and the high-altitude seedlings while the southern and the (southern) low-altitude seedlings were less or not inhibited. Too many seedlings died to permit assessing statistical significance of these interactions. Because the data show the same pattern as in earlier similar tests with many tree species (3), the interactions are considered to be real, and therefore to suggest the existence of inherent photoperiodic variation in this species.

The existence of differences in photoperiodic responses in ecotypes from the same latitude (but from different elevations) may be puzzling at first. It is easily explained if the photoperiodic response is considered as an indirect evolutionary adaptation to environmental factors other than the photoperiod itself (3, 4). According to this theory photoperiods act only as reliable trigger factors for timing of the growth and rest cycles. The environments at the two elevations, 6300 and 500 ft, of course are different in various ways. The optimal duration of yearly growth must be shorter in high than low elevations, and the ecotypes of high altitudes should evolve to become dormant at an early date when the days are still long.

3. *Pinus monticola*

Methods

Stratified seed of two sources (British Columbia, 51° N., and Oregon, 45°) were germinated in sphagnum moss under continuous illumination. The young seedlings were transplanted in jars containing a mixture of forest humus, sphagnum moss, and sterilized sand (ratio 1:4:5). Four jars of each source were kept for 3 months in growth cabinets under each of three photoperiodic treatments (VSD, SD, and LD) arranged as shown in Fig. 1. This arrangement provided the same amount of light, but photoperiodically different (4) effects under each treatment. The light (intensity 500 ft-c) was provided by cool, white fluorescent tubes supplemented (10%) by incandescent bulbs.

In addition, one set of seedlings was grown in a greenhouse where sunlight (June to August max. 10,000 ft-c) was supplemented continuously with light from fluorescent tubes (500 ft-c). The air temperature varied usually from 15° C at night to 30°, occasionally to 35°, during the day.

TABLE III

Growth of *P. monticola* seedlings after 3 months under different daylength treatments

Treatment (see Fig. 1)	Stem length, mm	Dry weight of top, mg	Total dry weight, mg	Tips dormant
VSD in cabinet	64	50	60	Yes
SD in cabinet	70	80	110	Yes
LD in cabinet	98	120	150	No
Continuous light in greenhouse	94	460	620	No

Results

Table III and Fig. 4 indicate that the seedlings of *P. monticola* possessed a marked photoperiodic response. Under VSD the seedlings soon became dormant and grew very little. Most buds of the seedlings were broad and blunt under VSD, but long and pointed under SD. The seedlings in the cabinets were not as healthy and vigorous as in the greenhouse. The light intensity of 500 ft-c may have been too low for normal growth of this species.

There was little difference between the seedlings of the two origins. In most cases the northern (British Columbia) seedlings grew slightly better. The southern (Oregon) seedlings grew definitely better under VSD (Fig. 3). Unfortunately, the roots of the northern seedlings became diseased under this treatment in three out of four replications, and it is not known whether the superiority of the southern seedlings was a normal or an accidental phenomenon. If it were normal, the interaction of photoperiod and seed source would indicate the existence of photoperiodic ecotypes in *P. monticola*. Such ecotypes exist in many other tree species (1, 3, 4).

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PLANTGEOGRAPHICAL INVESTIGATIONS OF THE TUNDRA VEGETATION IN THE SOVIET UNION[1]

BORIS A. TIKHOMIROV

Abstract

The tundra zone, including the forest tundra and the polar deserts, occupies about 15% of the entire surface of the U.S.S.R., or around 3 million square kilometers. If to this is added the area of open forests in the north, the territory covered will be about 35% of the Soviet Union. During the last decades, agriculture and stock raising have been introduced into the northern taiga and forest tundra, locally even into the tundra itself. It has therefore become necessary to study the northlands intensively from both the theoretical and practical viewpoints.

Preliminary studies had already been done during the 18th and 19th centuries, beginning with Lomonosov and his school, and were followed by a series of expeditions by great naturalists to the north. The main research, however, took place after the October Revolution, and it was first devoted to the western parts of the tundra, but during the last decades it has spread to include also the eastern, Asiatic, parts.

On the basis of theoretical research a number of flora manuals have been published, concerning the northern areas, and a series of detailed ecological maps have been edited.

On the practical side, intensive investigations have been performed on natural pastures, especially with regard to reindeer management: the development and growth of the lichens have been studied and methods to utilize and improve them have been devised; air-reconnaissance has been used to establish the extent of pasture and meadow land; the biology of the northern plants has been studied, especially in connection with long-day and solifluction conditions; the ecological relationships of the plants in different associations have been worked out; the history and dispersal routes of the arctic flora have been established; and so on.

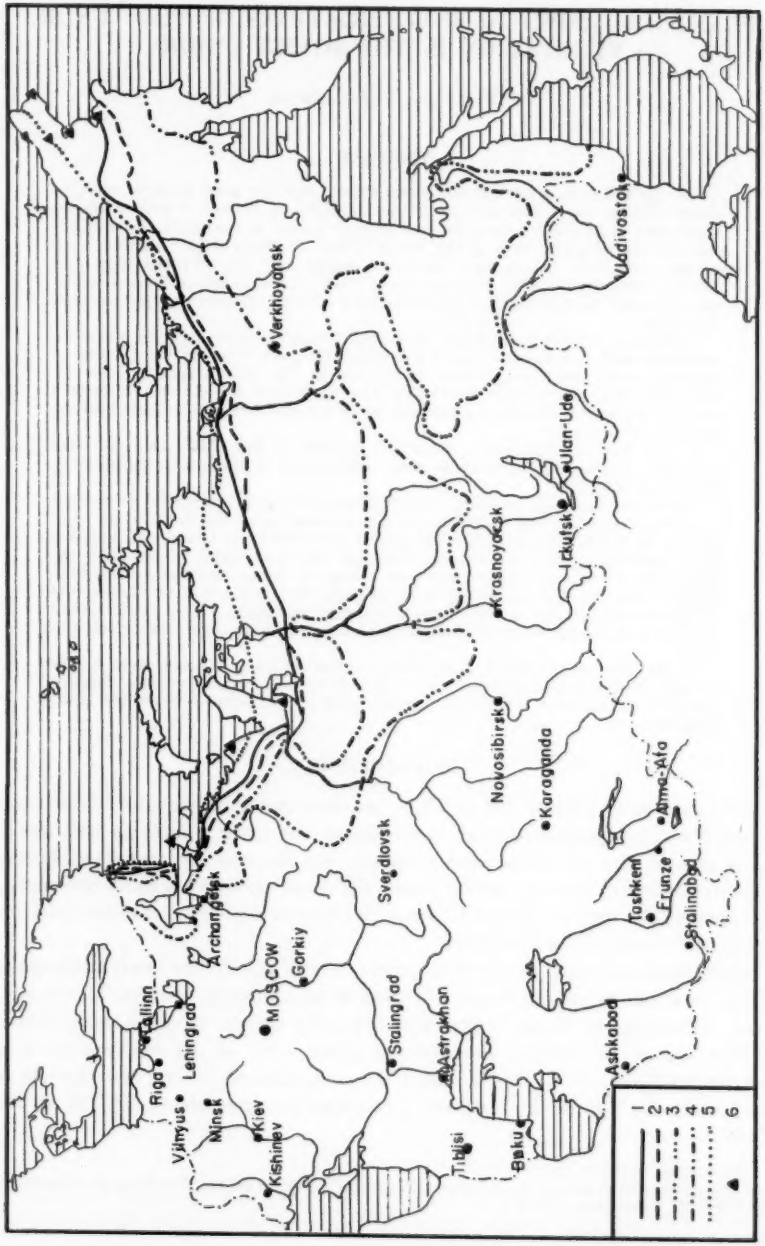
On the basis of this practical knowledge of the tundra conditions, there is now a trend to utilize and improve arctic agriculture, e.g., to enlarge and improve pastures and meadow lands, and to plant trees on the parts suitable for afforestation.

Introduction

The vast territories of the U.S.S.R. situated north of the continuous taiga forest (i.e., the forest parkland, the forested and open tundra as well as the polar desert) are of exceptional economic significance, because they border the Arctic Ocean, through which passes the great northern seaway connecting the ports of the western Soviet Union with those at the estuaries of the Siberian rivers and those of the Far East.

More than one third of the total area of the U.S.S.R. lies beyond the polar circle, and the ground in these districts is permanently frozen. The tundra zone, including the forest tundra and the polar desert, occupies more than 3 million square kilometers, representing about 15% of the entire surface of the Soviet Union. If to this is added the area covered by the open forests of the north, the size of the northern territories becomes about 35% of all the Union.

[1]Manuscript received June 17, 1960.
Contribution from the V. L. Komarov Botanical Institute of the Academy of Sciences of the U.S.S.R., Leningrad, U.S.S.R.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The U.S.S.R. shoreline along the Arctic Ocean is 25,000 kilometers long. The Arctic Ocean and the mainland south of it form a single natural unit, which possesses enormous natural resources, the study and utilization of which are of the greatest economic interest.

The native population of this territory has a very specific kind of occupation—nomad reindeer herding—but engages also in fishing as well as trapping and hunting of wild animals. Such a combination of human economic activities does not exist in any other part of the world outside the Arctic.

During the last decades, agriculture and the raising of domestic stock and fur animals have penetrated into the northern taiga and forest tundra in the U.S.S.R., locally even into the tundra itself, and the frontier of activity is moving rapidly northwards (Fig. 1). In this connection methods for utilizing the natural resources have become enlarged and more elaborate, including research regarding the quantity and supply of vegetation of the extreme north.

Besides, it is of great theoretical interest to become acquainted with the natural development of the vegetation of the north. The arctic nature is so peculiar, and the influence of the physicogeographical processes on its organic life so specific, that it is unique as a geographical zone in this world, and it is hard to say where on our globe we can find a complete analogy to this land.

In the U.S.S.R. the efforts of V. I. Vernadsky and his followers have brought about an investigation of the biosphere, which in its development is closely related to the physicogeographical composition of the earth, and is dependent on it at the same time as it imposes important alterations on its dynamics.

Special attention should be paid to the green cover of the earth, the phytosphere, which regulates so many of the processes going on in the near-earth stratum of the atmosphere and in the soilsphere. Thus occurs, for example, the heat exchange between the atmosphere and the geosphere (lithosphere) through the medium of the phytosphere, which in the Arctic in a quite peculiar manner regulates the action of the solar radiation on the soil surface as well as the radiation from there into the atmosphere.

In the high-arctic territories, other features of the ground are also, to a considerable extent, dependent upon the vegetation cover: the distribution, dynamics, structure, and thawing of the snow; the distribution and dynamics of freezing and thawing of the permanantly frozen ground; the erosion phenomena; the formation of patterned ground (thermocarst); the denudation and solifluction of the soil; and so on.

FIG. 1. Map showing the extension of agriculture and the limit of forests towards north. Redrawn from Tikhomirov (1956, Fig. 4, p. 35). 1. Present northern limit for agriculture in the U.S.S.R. 2. Present northern limit of the forest tundra. 3. Extension of agriculture in 1916. 4. Extension of agriculture in 1939. 5. Northern limit of trees during the postglacial hypsithermal period. 6. Points where, in experiments, vegetables have been grown successfully outdoors in the north.

Short Historical Review of the Botanical Research in the Far North of the U.S.S.R.

The vegetation of the northlands has long attracted the attention of botanists. The beginning of scientific research on the tundra zone of our country is connected with the names of the greatest naturalists of the 18th and 19th centuries. A number of fundamentally important ideas about the tundra were expressed by Lomonosov and several of his pupils and followers—Lepyokhin, Zuev, and Ozerietskovsky—but there was more profound research by the scientists of the 19th century. From the year 1837 on, footprints mark the trails of Baer to Novaya Zemlya and of Schrenk to the Bolshezemelskaya Tundra (Schrenk 1848, 1854) and, especially, of the famous Siberian expedition by Middendorf in 1843, who first of all revealed to the scientific world the specific nature of northern and eastern Siberia (Middendorf 1843–1847). The period of more than 100 years which has passed since these discoveries has been marked by various extensive and intensive research projects on the tundra vegetation of our country. The end of the 19th century and the beginning of the 20th witnessed some concentrated studies mainly of the western part of the Russian tundra (Kjellman 1883; Tanfiliev 1911; Pohle 1903, 1910, 1919; Palibin 1903–1906; Sokolovsky 1905; and others). Of great importance is the floristics and plantgeography of the Siberian seacoast as described by Kjellman (1883) on the basis of material collected during the "Vega" expedition. During the same period a first attempt was made to produce, with short descriptions, a phytosociological study of the Siberian vegetation from the lower reaches of the rivers Lena (Cajander 1906) and Yenisey (Kuznetsov 1916).

Up to the time of the October Revolution, the vegetation of the Russian Far North had been explored mostly in its western parts. The attention of botanists had mainly been concentrated on floristics, while plantgeographical research was fragmentary.

No research of any kind was done on the vegetation with the practical purpose of increasing the production of reindeer or the improvement of pastures. Only the last three decades have seen the development of a most intensive study of the tundra vegetation of the U.S.S.R. Below we will give a summary of the results achieved by a whole army of Soviet botanists working in the Far North.

Principal Results of Plantgeographical Investigations on the Tundra Vegetation

In the Soviet Union tundra management has appeared as a part of geobotany. With its essential ideas and terminology formulated on the basis of that science, it now comprises the study of the plant associations of the tundra zone against the background of their physicogeographical environment (Sochava 1931; Korchagin 1933; Gorodkov 1935*a*).

In 1930, for the first time in the history of university education in our country, B. N. Gorodkov began to give a special course in "tundra management" (cf. Gorodkov 1935*a*, 1938) from the chair of geobotany at the University of Leningrad, and now this course is included in the curriculum and offered by all universities, besides other special courses in geobotany. The general properties of the vegetation in the tundra zone of the U.S.S.R. having thus become established, it has become possible to put it on a map of the vegetation of the U.S.S.R. at a scale of 1:5 million, edited by Lavrenko (1939), and on a vegetation map of the European part of the U.S.S.R. at a scale of 1:2.5 million, edited by the Botanical Institute of the Academy of Sciences of the U.S.S.R. (cf. Lavrenko and Sochava 1948).

On the "Geobotanical map of the U.S.S.R.", made to a scale of 1:4 million (edited by Lavrenko and Sochava 1954), the vegetation of the tundra zone is plotted in even more detail than on the two previously mentioned maps. The text to this latter map, written by Sochava and Gorodkov (1956), is a considerable contribution to the plantgeography of the northern Soviet Union. See also the map by Sochava (Fig. 2), which in a simplified form illustrates the vegetation zones of the U.S.S.R.

These comprehensive geographical studies of the tundra vegetation have been made possible thanks to the discovery of the general laws of plant distribution as well as to the key maps and material prepared by numerous expeditions to different parts of the U.S.S.R. tundra.

During the first 15 years after the October Revolution, research work had been performed on the flora and vegetation of the tundra of Taimyr (Tolmachev 1932–1935; Shumilova 1933; Avramchik 1937; Vinogradova 1937), the Anadyr Basin (Sochava 1930, 1930*a*; Ovsyannikov 1930; Tyulina 1936; Vassil'ev 1936, 1956*a*; and others), and the Penzhina Basin (Sochava 1932; Gorodkov 1935*a*; Tikhomirov 1935; and others), as well as on the tundra of the European part of the Soviet Union (Perfil'ev 1934–1936; Sambuk 1930, 1931; Andreyev 1947; Dedov 1931, 1933; Gorodkov 1932, 1932*a*; Leskov 1937; Korchagin 1932; and others). Studies had also been made of the arctic islands Novaya Zemlya, Kolguiev, Vaigatch, Severnaya Zemlya, and Wrangel Island (Tolmachev 1930, 1931, 1936; Alexandrova and Zubkov 1937; Bogdanovskaya-Guinéneuf 1938; Korotkevich 1958; Gorodkov 1958; and others), Ural (Andreyev, Igoshina, and Leskov, 1935; Igoshina and Florovskaya 1939; Kildushevsky 1959; and others), as well as western Siberia (Gorodkov 1932; Govoruchin 1957; Leskov 1938; and others).

Recently, attention has been paid to a number of eastern regions, hitherto insufficiently known from a botanical point of view: the lower reaches of the river Lena (Gorodkov and Tikhomirov 1937; and others), the Tana Basin (Yarovoy 1939; Tyrtikov 1955), the Yenisey area (Kuznetsov 1952), the Indigirka region (Sheludyakova 1938; Tyrtikov 1958), eastern Siberia (Vassil'ev 1956), the area around the Chutkotka springs, and the Asiatic coast of Bering Sea (Tikhomirov 1957, 1957*a*).

Until recently, our botanists studied mainly the relatively southern regions of the tundra. The polar desert zone, mostly to be found on the islands of the Arctic Ocean, has been insufficiently attended to, though brief studies on the vegetation of the polar desert on Novaya Zemlya have been made by Alexandrova and Zubkov (1937). Thanks to the efforts of Gorodkov (1939, 1939*a*, 1947, 1958*a*) and Korotkevich (1958) during recent years, there are now descriptions of the vegetation of the polar deserts of Franz Joseph's Land, Severnaya Zemlya, the extreme northern part of Taimyr, Kotelny Island in the group of the Novosibirsky Islands, as well as of Wrangel Island. Alexandrova has investigated the vegetation of the Bolshoi Lyakhovsky Island, and Tikhomirov has for several years been studying the flora and vegetation of central and northern Taimyr (Tikhomirov 1948, 1948*a*).

Of exceptional importance for a thorough knowledge of the tundra vegetation was the inventory of natural pastures compiled under the guidance of Gorodkov (1933–1934). A study of the reindeer pastures was started in the late twenties under the leadership of Perfil'ev (1931–1933) on parts of the western tundra (cf. also Alexandrova 1937; Andreyev 1948), but since 1932 a large number of geobotanists have been at work in all parts of the U.S.S.R. tundra. As a result of these early works on reindeer pastures, as well as the inventory of the natural pastures of the tundra, methods for the utilization of the tundra were worked out for the first time (Sambuk 1931*a*; Dedov 1931, 1933; Sochava 1934), and an index of northern pastures (Alexandrova 1940) as well as a number of other works were undertaken. But in spite of considerable plantgeographical investigations having been made on different parts of the Far North of the U.S.S.R., there still remain little known areas, mainly in eastern Siberia, which are now the center of attention of our botanists (i.e., the territories from the lower reaches of the Lena to Chaunskaya Guba, the eastern coast of Taimyr, the Central-Siberian plateau, the Chersky, Verkhoyansky, Anadyrsky, Koriaksky, Gydan, and other mountain ranges).

Characteristic of all geobotanical research in our country is its practical aim (cf. Fig. 1). This is particularly so regarding the investigations of pastures for reindeer management. Since 1932, the exploratory work on vegetation suitable for reindeer pastures has been conducted systematically and is now managed on a grand scale at the stations of the Institute for Agriculture of the Far North (at Norilsk) or by special expeditions from the Ministry of Agriculture of the Russian Federation (Andreyev 1947). Specially worth mentioning are the permanent investigations by the Institute for Arctic Agriculture towards a knowledge of the regular development and growth of lichens on which to base a rational system for cultivation of reindeer pastures and particularly how to rotate these pastures (Andreyev 1940*a*). Methods for controlling the normal development and growth of the lichens have now been established and the geographical pecularities of their growth on different parts of the tundra zone have become known (Gorodkov 1936; Igoshina 1939; Nekrasova 1937, 1938; Sambuk 1936; and others). These questions have been studied during several years at the stations of the Institute for Arctic Agriculture, and the work has been reviewed by Andreyev (1954*a*).

A large number of geobotanists work on special land regulation expeditions from the Ministry of Agriculture of the Russian Federation and study large areas by the aid of aerovisual methods for the purpose of a correct organization and utilization of the reindeer pastures. The method of observation and investigation of tundra pastures from an airplane was invented in our country (Gorodkov 1935; Andreyev and Panfilovsky 1938; Andreyev 1940, 1955).

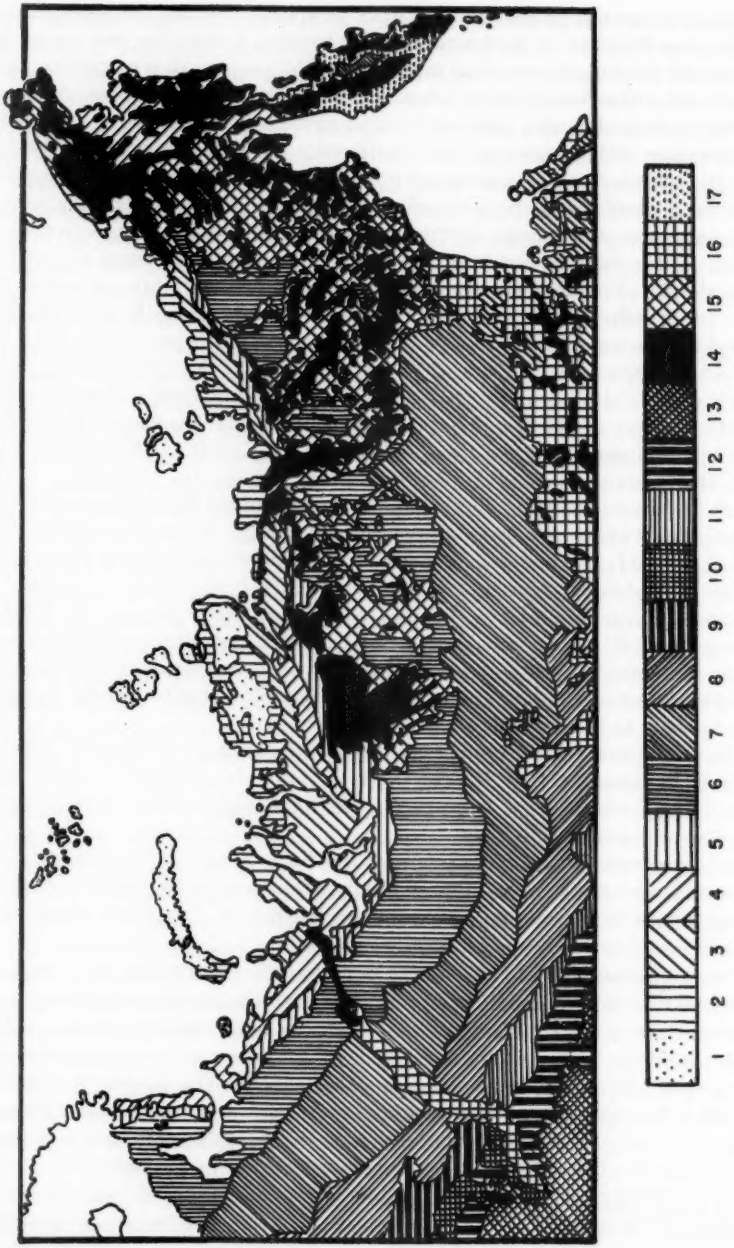
The basis for any plantgeographical research in the Arctic is the knowledge of the flora of the arctic territories. As a result of the exploratory work in various parts of the Soviet northland, a vast floristic material has accumulated. Part of this material has been used for a large number of notes and articles, the details of which I cannot afford time to deal with. I can only state that the plant collections from the northern regions of the U.S.S.R. have also been used for the compilations of flora manuals such as the "Flora of the U.S.S.R.," I–XXV (1934–1959), "Flora of Western Siberia," I–XI (1927–1937), "Flora of the Urals" (Govorukhin 1937), "Flora of the Far North" (Perfil'ev 1934–1936), "Flora of the Kamtchatka Peninsula," I–III (Komarov 1927–1930), "Flora of Kamtchatka and adjacent islands," I–IV (Hultén 1926–1930), "Flora of the Murmansk region," I–IV (1953–1959), "Conspectus of the flora of Yakutsk" (Karavayev 1958), "Flora and paleography of the Komandorsky Islands" (Vassil'ev 1957), as well as sundry local floras by Tolmachev (1932–1935), Leskov (1937), Tikhomirov (1948), Yurtzev (1959), and others. Thus, as has been shown, the systematic and floristic research in the Soviet northland has with each year accumulated new material for an arctic flora of the U.S.S.R. As a result it has become necessary to make a critical study of the arctic flora of the Soviet Union, and such a work is now being prepared by Tolmachev and his collaborators for publication by the Botanical Institute of the Academy of Sciences of the U.S.S.R.

It is also worth mentioning here the very important publication "Flora of the Cryptogams of the U.S.S.R." (I–II, 1952; III, 1954; IV, 1957; to be continued), which fills a large gap in our knowledge of this plant group, so important in the northlands.

The understanding of the origin of the flora as well as the evolution and the dynamics of the vegetation is hardly possible without a profound knowledge of the biological and ecological peculiarities of the plants forming the plant associations of the Arctic (cf. Fig. 2).

An extensive study of the nature of the arctic plants gives us a basis for judgment of their possibilities of adjustment to the specific conditions of the north. Such a work towards the knowledge of arctic plants is being tackled from different angles in the U.S.S.R. For instance, an investigation of the underground parts of arctic plants has been made. The papers by Dadykin (1954), Tyrtikov (1951), Karandina (1954), and others have given us basic information on this problem and demonstrate the ability of the root systems to adjust to a limited space in the thawing layer of the ground.

A special place in the study of plant biology is occupied by research on the biological development and rhythm of flowering and fruiting. Evidence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gathered by Soviet tundra specialists regarding the condition of plants under the snow cover in the north (Avramchik 1939; Alexandrova 1940, 1959; Glinka 1939; Kildyushevsky 1956; Temnoyev 1939; Tikhomirov 1956*b*; and others) has been of great interest for plant biology, but has also given completely new ideas regarding winter feeding of animals in the Arctic.

Observations of the developmental rhythm of arctic plants, carried out during recent years, demonstrate that a large variety of plant taxa have adjusted to short vegetative periods, and have also given sufficient proof of the quickly changing aspects of the tundra plant associations (Alexandrova 1959; Tikhomirov 1956*a*; and others). I cannot but mention the unique research conducted in the Soviet Arctic on the rhythmic development and biology of plants under the specific 24-hour long-day conditions of the northlands (Shamurin 1958).

Of great importance also is the work revealing the peculiar interrelationship between different biological groups in the arctic plant associations. Especially important for the existence of vascular plants and lichens is the role of the mosses (Tikhomirov 1952). As a number of investigations have shown, there is no doubt about the enormous role played by algae in the formation of vegetation cover on a substrate, particularly on the barren spots so often occurring in the tundra (Kosheleva and Novichkova 1958; Dorogostayskaya 1959; and others).

Though very little is known about mycorrhiza on arctic plants (Tikhomirov and Strelkova 1954; Strelkova 1956) or about the existence of bacteria in the arctic Leguminosae (Kriss 1947, 1957), these problems are without doubt of great interest in understanding the essential interrelationships in the plant associations.

In connection with the research work on pastures for reindeer and other animals, problems of the biological and economical productivity of the tundra plant associations have been brought forward. As a result information has been obtained on the accumulation of organic matter in different tundra associations (Andreyev, Igoshina, and Leskov 1935; Andreyev 1954; Govorukhin, 1950; Lavrenko, Andreyev, and Leont'ev 1955; Alexandrova 1958).

The knowledge of the habitat of the arctic plants in their natural surroundings seems of great importance in conditions of the Far North. The character of the soil (cf. Gorodkov 1939*a*, 1947, 1958) and the permafrost, as well as the dynamics of the vegetation in connection with the thawing of the fossil ice in the north, has been the subject of several investigations by Gorodkov (1932, 1939*a*), Liverovsky (1934), Tikhomirov (1959*a*), Dorogostayskaya (1959*a*), and others.

FIG. 2. Vegetation zones of the U.S.S.R. Redrawn from a color map by Sochava (1954); northern part only. 1. Arctic glaciers and polar deserts. 2. Arctic tundra. 3. Typical moss–lichen tundra. 4. Shrub- and bush-tundra. 5. Open forests on semitundra (sylvotundra). 6. Northern taiga. 7. Central taiga. 8. Southern taiga. 9. Conifer forest with deciduous elements. 10. Deciduous, broad-leaved forest. 11. Small-leaved (parvifoliate) forest. 12. Bush-steppe. 13. Typical grass-steppe. 14. Alpine tundra. 15. Open montane forest of northern taiga type. 16. Montane forest of central and southern taiga type. 17. Open subalpine birch forest, and the herbaceous vegetation of the subarctic islands.

Among the factors which influence the dynamics of the tundra vegetation, the animals occupy an important place, and their relation to the vegetation has recently been investigated by Tikhomirov (1959).

The Soviet botanists have discovered the main lines in the history of the formation of the flora and vegetation of the Soviet Arctic (cf. Sochava 1944; Tikhomirov 1941*a*). Our evolutionary–genetical approach has proved a particularly beneficial basis for the study of the history of the formation of the flora and vegetation of the Arctic. First and foremost I would like to mention some investigations regarding the question of the origin of the arctic flora and its separate genetical elements (Tolmachev 1930, 1932–1935; Gorodkov 1939; Sochava 1944; Kats 1944; Tikhomirov 1946; and others). In these papers the main routes for the formation of the floristic complexes in the high north are explained. No less attention has been paid to the origin and development of the vegetation in relation to the formation of the tundra landscape (Tolmachev 1927, 1944; Tikhomirov 1941, 1944, 1946, 1954*a*; Gorodkov 1952; and others).

Finally, I want to mention the discussion regarding the origin of the different plant associations which are widespread in the northeastern parts of the U.S.S.R. (Tikhomirov 1949, on *Pinus pumila*; Kolesnikov 1937, and Norin 1958, on *Chosenia macrolepis*; Vassil'ev 1941 on *Betula Ermanii*; etc.).

The Soviet botanists have for a long time paid special attention to the question of relationship between the forest vegetation and the tundra, and to the reason why the tundra is treeless (Gorodkov 1929; Tyulina 1937; Sochava 1940; Medvedev 1952; Tikhomirov 1953, 1954*a*; Tikhomirov and Shtepa 1956; Andreyev 1956; and others). Now the main causes for the absence of trees on the tundra are known, and on the basis of this, methods for afforestation have been suggested. We have also revealed the present tendency of a northward and vertical expansion of the vegetation (Tyulina 1936, 1937; Tikhomirov 1941, 1953, 1954*a*; Galazy 1954; Andreyev 1954*b*). These last investigations are of essential significance for the transformation of the tundra and the increasingly effective and economical utilization of it. On the basis of the known normal conditions, serious attempts have been made to interfere with the natural processes of the tundra.

Experiments are being made to convert the tundra into grassland (Dydina 1954) and artificial meadows, to drain waters and lakes and create meadowland on their bottoms (Pryanishnikov 1954, 1955), to regulate the bush-growth on floodlands along the rivers so as to increase the productivity of the meadows and pastures (Savkina 1951), and to plant trees on the tundra (Andreyev 1954).

As a result of the acquaintance with the natural tundra vegetation, works containing general conclusions of great theoretical value have been published. Worthy of being mentioned among them are the review by Gorodkov (1935*b*, cf. also 1948) on "The vegetation of the tundra in the U.S.S.R.", the books by Grigor'ev (1956) on the "Subarctic", the chapter Tundra in the book by Berg (1930) on "Landscapes and geographical zones of the U.S.S.R.," and others.

The present article, though far from being complete and exhaustive, attempts to review the work done by Soviet botanists and specialists on the tundra, and to show the broad scope of the research directed towards the mastering of the natural tundra meadows, their flora, and their vegetation. It was prepared for and delivered to the IXth International Botanical Congress in Montreal in August, 1960.

Acknowledgment

The list of references following below has been made in co-operation with Miss C. Dorogostayskaya, V. L. Komarov Botanical Institute, to whom I express my gratitude.

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"STEREUM PINI" IN NORTH AMERICA[1]

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Abstract

Interfertility tests between American "*Stereum pini*", European "*Stereum pini*", and American *Peniophora duplex* have shown that a closer relationship exists between the latter two taxa than between them and the first-named taxon. This genetic relationship is supported by their morphology in non-fruiting culture though not by the morphology of their natural fructifications. It is concluded that the three taxa are best treated, at present, as *Peniophora pini* aggregate, composed of *P. pini* (Schleich. ex Fr.) Boidin subsp. *pini* (of Europe), *P. pini* subsp. *duplex* (Burt) stat. nov., and *P. pseudo-pini* nom. nov. (both of North America).

Introduction

When Nobles (1956) published her study of conifer-rotting *Peniophora* species of the Coloratae group, she included (*a*) isolates from decays and fruit bodies which had been determined as *Stereum pini*, and (*b*) Unknown Fungus "T", which had been isolated from decays and remained unidentified because it had been shown, in interfertility tests, to be blocked from all the others by a complete sterility barrier. While the paper was in press, Dr. Nobles and the junior author of this paper were successful in collecting a number of fruit bodies which yielded monospore isolates capable of producing clamped mycelium when paired with haploid cultures of Fungus "T". These fruit bodies were determined by Nobles as *Peniophora duplex* Burt; and her identification has been confirmed through comparison with the type specimen borrowed from Farlow Herbarium.

P. duplex was described by Burt ([1926] *a*) on the basis of a single collection on cultivated *Pinus austriaca*, from Shelter Island, N.Y. The later-described *Corticium overholtsii* Burt, based on a collection on *Pinus rigida* from Pennsylvania, was placed in synonymy with *Peniophora duplex* by Rogers and Jackson (1943). At the same time, they referred to the similarity in structure of *Stereum pini* and *P. duplex*, and suggested that the latter might be "a growth phase or variety" of the former, a relationship to be clarified by "further study, involving cultures".

The work reported by Nobles in 1956 and continued here is this further study. But with the receipt of freshly collected fruit bodies of *S. pini* from Europe, generously supplied by Dr. Eriksson from Sweden and by Prof. Boidin from France, our considerations have spread to include not only the problem of the relationship between *S. pini* and *P. duplex*, but also of that between *S. pini* of Europe and *S. pini* of America, as well as an assessment of the arguments regarding the generic affiliation of these taxa.

[1]Manuscript received April 25, 1960.
Contribution No. 77 ffom the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

Stereum pini (Schleich. ex Fr.) Fr. has been reported in Europe and Asia from as far north as Finland and Sweden, south to France and Bulgaria, east to Mongolia, and west to Britain; and in North America's temperate areas, as far west as British Columbia, east to Ontario, Maine, and New York State. Its identity has gone unquestioned. Overholts (1930) found an "aberrant" collection which he first named *Phlebia cervina*, but he soon (1939) reduced that name to synonymy with *S. pini*. Pilát (1930) labelled as f. *minor* a collection of fruit bodies sent by Maire "welche durch ihre enorme Grösse sehr auffallend waren". And, as mentioned above, Rogers and Jackson indicated a possibility that *Peniophora duplex* might belong in the same species. Throughout its long history since Fries's adoption of the name "*Stereum pini*", these seem to have been the only moments of doubt regarding the characteristics and circumscription of this taxon.

The question of its correct generic place has, however, been under discussion for some time. It has been generally agreed that *S. pini* does not belong in a genus typified by *S. hirsutum* (Boidin 1958*a*, and others). Since Wakefield's (1948) comment on *S. pini's* place among the Coloratae *Peniophorae* (i.e. *Peniophora* sensu stricto or, perhaps more correctly, *Corticium* s.s.: cf. Rogers 1949, Eriksson 1950, Donk 1957*a*), Nobles (1956) has demonstrated the closeness of the relationship with cultural studies, and Boidin (1956) has made the logical transfer to the genus *Peniophora*, stating: "il s'agit notamment de la couleur des spores, des gloeocystides sulfo-aldéhydes positives, de la disposition hémi-chiastobasidiée des fuseaux mitotiques de la baside", with a later (1958*a*) comment: "*Stereum pini* . . . a tous les caractères des *Peniophora* sensu stricto (spores roses, gloeocystides sulfo-aldéhydes positives) . . .".

But the problem was not settled. Donk (1957*b*) proposed, as of possible value, the revival of Karsten's genus *Sterellum*, typified by *S. pini*, and Eriksson (1958) concurred by adopting this "satellite" genus for the sake of a more homogeneous *Peniophora*, the former differing from the latter "especially in having gelatinous hypha walls and white spore print".

Our contribution to the discussion on this point is presented below, with our evidence and conclusions. Until these have been placed on record, we shall refer to the taxa involved as European *Stereum pini*, American *S. pini*, and *P. duplex*.

Techniques

Squash-mounting of minute portions of hymenium or mycelium in ca. 7% KOH and phloxine is a well-known technique. The addition of Congo red in 10% ammonia was recommended to the senior author by Dr. R. J. Bandoni, who has recently (1958) reported using it, and its value has been well described by Boidin (1951*a*, 1958*b*). It was Boidin's (1951*b* etc.) work with sulphuric-aldehydes that introduced us to the use of sulphuric-benzaldehyde, which we have found of immeasurable assistance for the recognition of gloeocystidia even in very old herbarium collections of Coloratae. And we, like Boidin (1951*a*), have found that it is possible to observe many characteristics under phase contrast with far greater ease than by ordinary bright-field microscopy.

In addition to squash mounts, sections of sporocarps have been used in our observations. Since these sections had to be thick enough (not "zu dünn", cf. Overholts 1929*a*) that gloeocystidia 20–30 μ in diameter might be left intact, it has been satisfactory to make rough hand-sections while viewing the sporocarp through a dissecting microscope, whereby the precise relationship of section to fruit body might be noted—radial, tangential, through umbo or margin, etc.

The methods used in culturing these fungi and in describing the cultures have been completely reported by Nobles (1948), and the key patterns given below are in accordance with her studies. Interfertility tests were conducted in the manner described by many authors (Mounce and Macrae 1936; Macrae 1942; etc.): spore prints were collected on glass slides, spore dilutions made on agar plates, germinating single spores isolated for culturing, and transfers from the resulting monosporous mycelia made to malt agar slants in pairs, about 2.5 cm apart, their mingled hyphae being checked in about three weeks for the presence of clamp connections. In a few cases, replicates were made on plates so that the distribution of clamps might be noted—whether throughout the plate, or localized at the point of meeting of the inocula, or in association with one or other of the two.

Where colors are referred to a color standard, the Munsell Book of Color (Munsell Color Co. Inc., Baltimore, Md. 1929–1942) has been used. Herbaria are designated in accordance with the abbreviations published in the Index Herbariorum (The Herbaria of the World I, 4th Edition, Regnum Vegetabile **15**, 1959).

Interfertility Studies

In Table I are listed the collections from which monospore isolates were made for use in our interfertility tests. Our herbarium numbers (DAOM) are given, as well as whatever collectors' numbers we have: e.g. in the case of the material sent us by Prof. Boidin, his Lyon numbers are noted, LY *3181* being a large collection of sporocarps he collected especially for us, LY *1999* and LY *1779* his own monospore isolates from other collections.

Figs. 1–4 give the results of the tests, and are here first explained separately, then discussed together and summarized in Fig. 5. It is because our data are not of the decisive yes-or-no nature usually obtained in pairing tests that it is felt necessary to give and discuss them in detail.

Figure 1

When Nobles (1956) distinguished between *S. pini* (American) and Unknown Fungus "T", the tests represented by the pairing tables in Fig. 1 had been made. American *S. pini* (DAOM *22982*, *30124*, *30466-A*, and *30521*, as well as others not reported here) and Fungus "T" (DAOM *31229* to *31233* inclusive) showed no sign of being able to mate with each other. *S. pini* (American) itself, in the self-mating of DAOM *22982*, showed a normal tetrapolar behavior, as did Fungus "T" in the self-mating of DAOM *31232*.

TABLE I

Cultures used in interfertility studies

DAOM No.	Source	Host	Locality	Collection date
Peniophora duplex				
31229	Rot	*Pinus banksiana*	Sudbury D., Ont.	1951
31230	Rot	*Pinus banksiana*	Sudbury D., Ont.	1951
31231	Rot	*Pinus banksiana*	Sudbury D., Ont.	1951
31232	Rot	*Pinus banksiana*	Sudbury D., Ont.	1951
31233	Rot	*Pinus banksiana*	Sudbury D., Ont.	1951
31784	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31785	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31787	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31788	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31789	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31790	Spores	*Pinis banksiana*	Petawawa, Ont.	1955
31791	Spores	*Pinus banksiana*	Petawawa, Ont.	1955
31792	Spores	*Pinus resinosa*	Petawawa, Ont.	1955
53383	Spores (Slysh *597*)	Conifer	Pack Forest, N.Y.	1958
53384	Spores (Slysh *611*)	Conifer	Pack Forest, N.Y.	1958
American *Stereum pini*				
22982	Spores	*Pinus resinosa*	Simcoe Co., Ont.,	1951
30124	Spores	*Pinus strobus*	Blakeney, Ont.	1952
30466-A	Spores	*Pinus sylvestris*	Simcoe Co., Ont.	1953
30521	Spores	*P. contorta* v. *latifolia*	Water Valley, Alta.	1953
31793	Spores	*Pinus banksiana*	N. of North Bay, Ont.	1955
31794	Spores	*Pinus banksiana*	N. of North Bay, Ont.	1955
31795	Spores	*Pinus banksiana*	N. of North Bay, Ont.	1955
31796	Spores	*Pinus banksiana*	N. of North Bay, Ont.	1955
European *S. pini*				
53381	Spores	*Pinus sylvestris*	Värnamo, Sweden	1958
53382	Spores (LY *3181*)	*Pinus sylvestris*	Rhône, France	1959
53491	LY *1999*	*Pinus sylvestris*	Sainte-Peray, France	1954
53492	LY *1779-B*	*Pinus sylvestris*	Rhône, France	1955

In the latter case, it is perhaps noteworthy that the first readings of pairings between monosporous cultures *5* and *10*, *5* and *18*, *15* and *9*, *15* and *14*, and *17* and *4*, were scored as positive; but a re-reading showed no clamps: apparently a case of illegitimate pairing that was rare or unstable, or of incompleted clamps misread as true clamps.

It was in pairings between monospores of different isolations of Fungus "T" that a partial compatibility was observed. In these crosses, when four monosporous mycelia of each of the isolations from decays which had fruited in culture, DAOM *31229* to *31233*, were paired in all combinations with four monosporous mycelia of each of the others, many pairings did not yield clamped mycelium. In fact, only one of the sets of pairings (*31230* × *31233*) was even 50% successful. It was possible, using the crosses between monospores from isolates from four trees growing within a single study plot (DAOM *31230* to *31233*) to construct a fairly satisfactory tetrapolar table (reproduced from Nobles in this figure), designating identical incompatibility alleles as common to these different isolates.

These were the data presented by Nobles in 1956.

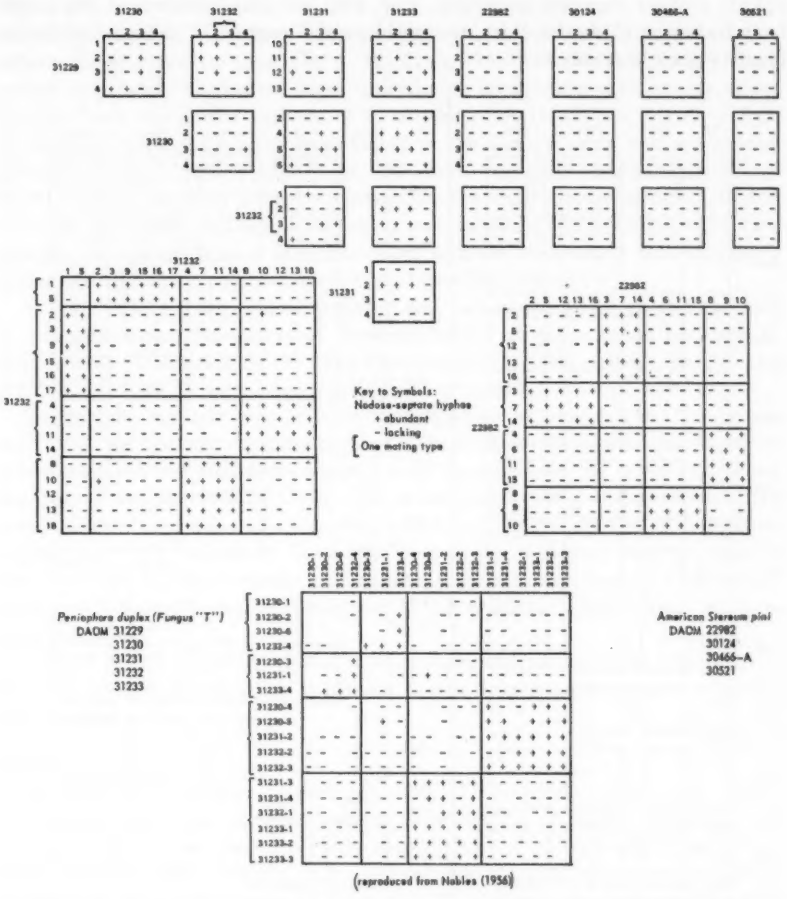


FIG. 1. Results of inter- and intra-pairings, using *Peniophora duplex*, isolated from decays in 1951, and American *Stereum pini*, isolated from fruit bodies in 1951–53; tests conducted in 1952–54.

Figure 2

Tests with the 1955 collections (cf. Table I) confirmed the earlier decision (Nobles 1956) that the collections assigned to Fungus "T" belonged together and were separate from *S. pini* (American). The North Bay collections (DAOM *31793* to *31796*) proved to be completely compatible with *S. pini* (American) (DAOM *22982* and *30521*), and completely incompatible with Fungus "T" (DAOM *31229* and *31232*). Furthermore, every pairing between the latter two isolates and the collections from Petawawa (DAOM *31784* to

31792) yielded clamped mycelium. And with the identification of the latter fruit bodies as *P. duplex* Burt, the problem of Fungus "T", at least as far as identity goes, was solved.

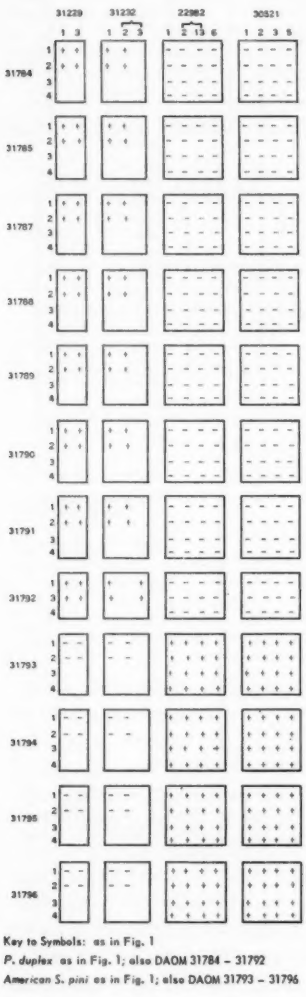


FIG. 2. Results of pairings made in 1955, using isolates from freshly collected fruit bodies of *Peniophora duplex* and American *Stereum pini*, as well as 1951–53 material.

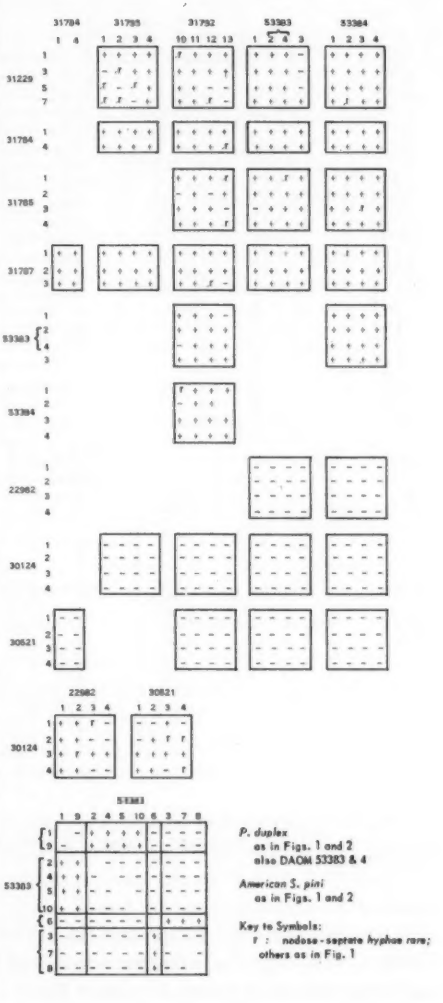


FIG. 3. Results of pairings made in 1958–59, using isolates from freshly collected fruit bodies of *Peniophora duplex*, and earlier collections of *P. duplex* and American *Stereum pini*.

Figure 3

Further confirmation came with pairings conducted on the arrival of two collections of *P. duplex* from Dr. A. R. Slysh, DAOM *53383* and *53384*. They paired completely with each other, and almost completely with monosporous mycelia from the earlier isolates of *P. duplex*—DAOM *31229*, *31784*, *31785*, *31787*, and *31792*. The latter were tested at the same time in pairings among themselves, and gave evidence of less than complete compatibility: e.g. *31785* × *31229* showed a normal picture of consistent clamps in only half the pairings. In these readings, a notation was made of the occurrence of rare clamps (r) amidst mainly simple-septate hyphae, because it was necessary to distinguish between such a result and a complete pairing (+).

In spite of the scattering of negative and rare-clamp pairings, the contrast is sharp between the results of these tests and of those made between *P. duplex* and collections of *S. pini* (American) (DAOM *22982*, *30124*, and *30521*), all of which were negative without exception.

To test the maintenance of their mating capacity through the 5 years in culture of the last-named collections of *S. pini* (American), four monosporous mycelia of DAOM *30124* were paired with four each of the other two. They had paired completely in 1953; in 1958, as the tables in Fig. 3 show, some of the pairings were negative and some with only rare clamps, like the 1958 pairings among the 1953 isolates of *P. duplex*. But again, sufficient mating capacity was retained to demonstrate clearly the intimate relationship within each group of tested collections, in contrast to the consistent negatives of matings between them.

DAOM *53383* was selfed, giving a further demonstration of the tetrapolarity of *P. duplex*, and supplying a full set of the four mating types for further consideration.

Figure 4

Material of European *S. pini* (DAOM *53381*, *53382*, *53491*, and *53492*) broadened the scope of our problem. The selfing of *53381* confirmed the tetrapolarity of the taxon, already recorded by Tassinari (1956) using LY *1999* (DAOM *53491*) (Boidin, in litt.). But in the pairings between monosporous mycelia of these collections of European *S. pini* and the test monosporous mycelia of American *S. pini* (DAOM *22982*, *30124*, and *30521*) not one clamp connection was observed.

On the other hand, pairings of European *S. pini* with test monosporous mycelia of *P. duplex* (DAOM *31229*, *31784*, *31785*, *31787*, *31792*, *53383*, and *53384*) yielded abundant evidence of a close enough relationship for the production of a common nodose-septate mycelium. The most successful pairings were between *53381* and *53384*, one set showing complete compatibility; but a later replicate and a different set of pairings showed only partial compatibility between them. Matings between *53381* and *53383* showed a similar inconsistency. Here, since in both collections self-pairings had sorted out their mating types, it was possible to compare the reaction of monospores judged to be of the same mating type (in "*ab*" terms): *53383-2* and *53383-4* were

slightly different in reaction in the first set, but both consistently negative in the expanded set of pairings made later; *53383-1* and *53383-9*, however, reacted as though they were quite different. There was, perhaps, a somewhat better correlation between pairs of *53381* (e.g. spores *1* and *4*), but little assurance of dependable uniformity of action. Pairings of European *S. pini* with the earlier (1955) collections of *P. duplex* were even less dependable than with the 1958 collections of this taxon, and yielded fewer matings that could be considered completed.

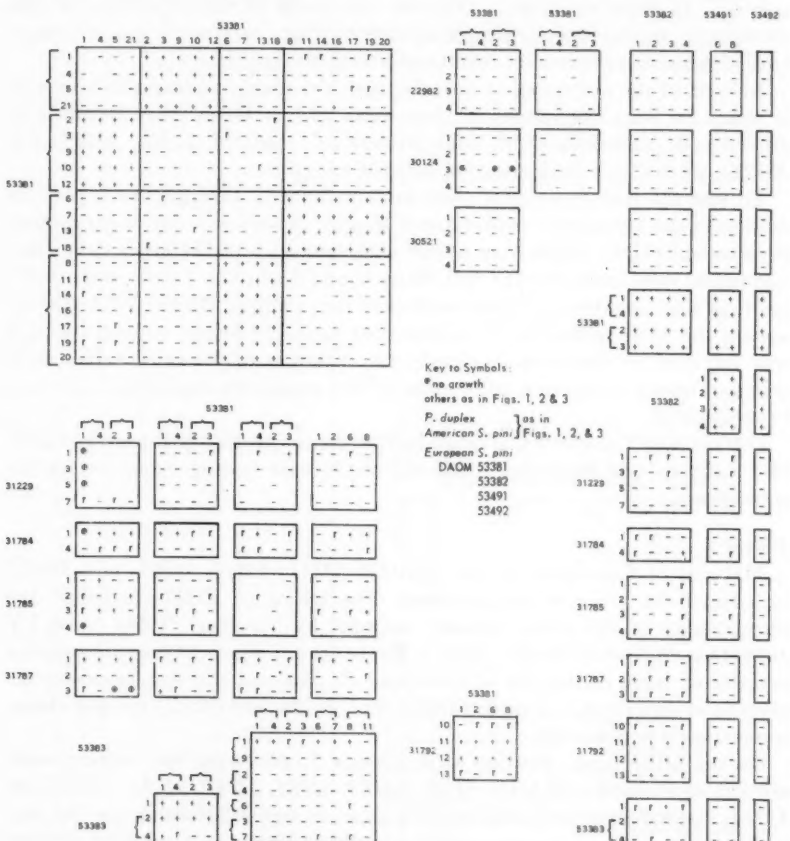


FIG. 4. Results of pairings made in 1958 and 1959, between European *Stereum pini*, American *S. pini*, and *Peniophora duplex*. Identical tests are arranged from left to right in chronological order. Vertical rows are not necessarily indicative of simultaneous testing.

However, between monosporous mycelia of the various isolates of European *S. pini* (DAOM *53381*, *53382*, *53491*, and *53492*), there was perfect pairing throughout.

Discussion (*Figs. 1–5*)

The foundation of specific identification through interfertility tests has, in the past, been the repeated demonstration that, as Nobles (1948) has said, "in heterothallic species, every monosporous mycelium obtained from one fruit body of a given species will pair with every monosporous mycelium from another fruit body of the same species from a different source so as to produce mycelium bearing clamp connections".

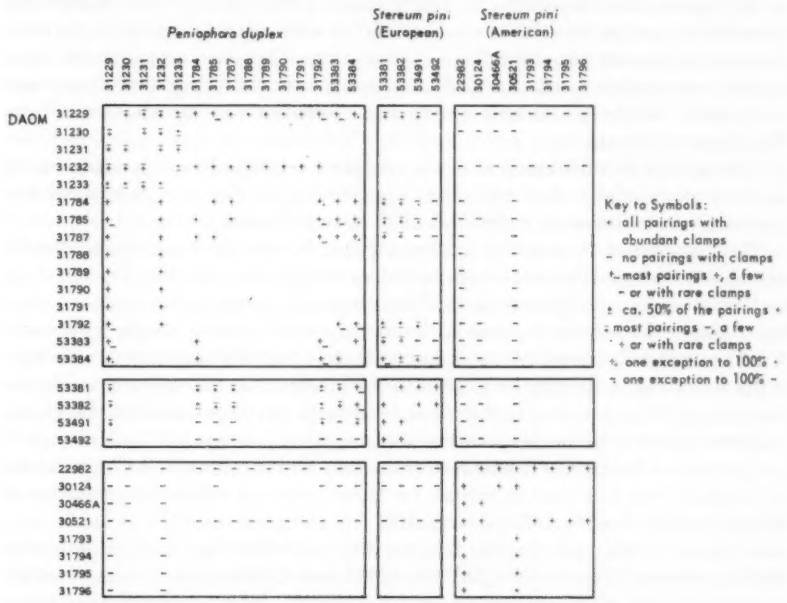


FIG. 5. Results of all pairings included in this study. Each symbol represents a summary of all pairings between monosporous cultures from a pair of collections. Note: the number of pairings per symbol is not necessarily the same in every case.

Such complete pairing exists within each of the two intrafertile and intersterile groups of European *S. pini* and American *S. pini*. This can be taken to be established in spite of the reduced compatibility that was demonstrated in the older cultures of American *S. pini* (Fig. 3: *30124* × *22982* and × *30521*), a reduction in pairing vitality that has been shown by other workers (e.g. Lange 1952, p. 68) to manifest itself in various ways.

These two intersterile taxa have, until now, been called one species. But there is no mating between them. Granted that we have had too few collections from Europe to evaluate the state of this species on that continent, we must

still explain the observation that there has been no sign of a clamp connection in pairings between collections from North America and collections from Europe.

At this point, this is not comparable to the problem in *Fomes pinicola*, in which Mounce and Macrae (1938) found a pronounced (though not complete) intersterility between two North American groups, one of which was highly interfertile with a third group of foreign origin, the other somewhat less so. In the case of the *S. pini* pair of taxa, we have found no linking group, American or European.

This is also not comparable to the problem of *Peniophora tenuis*, in which Boidin (1950) discovered two intersterile groups and one homothallic taxon, or of *Coprinus subimpatiens*, in which Lange (1952) found three completely intersterile groups, in neither case correlated with either geographic distribution or differences in morphological characters. Our case seems to be more closely comparable to that of *C. disseminatus*, in which Lange (1952) retained an Indian isolate intersterile with, but "identical in all characters" to, European material.

It is against this background of the complete intersterility of geographically isolated populations that we made our studies of the morphology of the American and European collections of *S. pini* (cf. below).

The question of *P. duplex* is a different one. Within the taxon itself, Nobles found evidence of identical alleles in different isolations (cf. Fig. 1), but these isolates were from so limited an area that they might have arisen from invasion by a single mycelium or been initiated by spores from a single fruit body (Nobles 1956). The negative pairings and those which showed only rare clamps, reported in Fig. 3, may be ascribable to the same cause, but we have made too few pairings with known mating types to come to any conclusion on this point. Another explanation of the occurrence of negative pairings and rare clamps—and perhaps a better one in this instance—may be the reduced mating capacity of material that has been in culture for three years, as witness the pairings of DAOM *31229-3* with *31785-1* and *31785-2*, complete in 1955 (Fig. 2), but negative and with rare clamps, respectively, in 1958 (Fig. 3). Certainly the freshly collected fruit bodies (DAOM *53383* and *53384*) gave no sign of either identical alleles or a reduced intercompatibility, pairing completely when crossed with each other (Fig. 2) as good conspecific isolates from different fruit bodies should.

That *P. duplex* is genetically separated from American *S. pini* is undeniable from the results tabled in Figs. 1–3 and summarized in Fig. 5. But what is the relationship between *P. duplex* and European *S. pini*?

In tests conducted simultaneously with intra-*pini* pairings that were wholly positive (Fig. 4) and intra-*duplex* pairings that were highly successful (Fig. 3), there was only one case (Fig. 4: *53381* × *53384*) of a completely successful set of pairings between *P. duplex* and European *S. pini*. Other pairings between them (Fig. 4) were frequently negative or with only rare clamps; and no correlation with mating type was found. Lange (1952) found cases of partial fertility which he attributed to "special factors [other than mating type]

reducing compatibility", but to which he assigned no particular taxonomic significance because there was "no connection with . . . geographical distribution". But in the case of *P. duplex* and European *S. pini*, we have two taxa which are geographically separated and demonstrate both reduced compatibility and inconstancy of pairing interaction: a combination that cannot be ignored in our classification.

As Fig. 5 shows in summary, there are three intrabreeding taxa under discussion. Of these, only American *S. pini* is completely cut off from genetic combination with the others. Since it is clearly possible to encounter a hymenomycete species within which the distribution of identical alleles can provide for less than 100% compatibility between different fruit bodies (cf. Mounce and Macrae 1937; Fries and Jonasson 1941; Whitehouse 1949; Lange 1952), we have already had to modify our demand for the successful mating of every pair of monospore isolates from different fruit bodies of what we judge to be the same species. A measure of intrasterility not due to common alleles is also being accepted as possible within a species (Lange 1952). Therefore, our three taxa may be considered members of an aggregate (Manton 1958) or a collective species (Biggs 1937) or a species complex, within which we have been able to recognize one population (American *S. pini*) intersterile with its sympatric fellow, *P. duplex*, and its allopatric counterpart European *S. pini*, while the other two allopatric populations have retained some measure of intercompatibility. The three populations might be assigned subspecific status within one species. Or, in recognition of the reproductive isolation of American *S. pini*, it may be separated as a distinct species. Or, taking this isolation into consideration, and the fact that *P. duplex* and European *S. pini* are far from normal in their interpairing, it might be more reasonable to designate each as a separate species, assuming that the compatibility between *P. duplex* and European *S. pini* is evidence for interspecific "hybridization", which has till now not been convincingly demonstrated as occurring in the homobasidiomycetes.

It must be remembered that our genetic evidence goes only as far as the observation that there is (or is not) a possibility for individuals to unite in the formation of a nodose-septate mycelium. Since the conditions for reliable fruiting in culture of these groups of fungi are unknown as yet, we are not aided in deciding on conspecificity or interspecific hybridization by any information regarding the ability of these pairings to culminate in a fruit body or fertile spores. From pairings on petri plates, we have found that some of the "fertile crosses" between *P. duplex* and European *S. pini* yielded clamps only at the line of meeting of two inocula, as in some cases of "illegitimate" pairings within a species (Lange 1952; Takemaru 1959; Macrae unpublished); and some were unilaterally dicaryotized. In the few serial transfers made of the clamped mycelium of these "fertile crosses", the hyphae eventually became simple-septate. It does seem that the dicaryon we assume to have been established is less stable in the erratic "fertile crosses" and cases of rare clamps in matings between *P. duplex* and European *S. pini* than in fertile crosses within a good species. If dedicaryotization takes place regularly,

these crosses can hardly be considered truly fertile, and do not therefore provide good evidence for conspecificity. However, further study of these matters is needed, including a simultaneous comparison with the results of "illegitimate" intraspecific pairings (e.g. Fig. 4: *53381* self-mating: monospores *21* × *16*, *2* × *18*, etc.), before conclusions can be based on such information and a denial made of such statements as Lange's (1952, p. 136): "A positive result of confrontation of two strains is . . . conclusive evidence of specific identity".

With this background of knowledge and ignorance of the intermating of these taxa, we proceed to the consideration of their morphology, and the decision regarding their classification.

Sporocarp Features: Observations and Comparisons

Spore Print

Eriksson's (1958) emphasis on color of spore print in segregating *S. pini* and *S. rufum* from Coloratae *Peniophorae* turned our attention only recently to the necessity of using white paper as a background for spore prints of members of this group, since on glass even the rosiest of Coloratae prints is barely discernible as tinted. Therefore only our most recent collections have been properly set up to reveal the color of the spore print. But in every case of a white-backgrounded print of European *S. pini*, American *S. pini*, *P. duplex*, and *S. rufum*, there was at least a faint salmon tint observable. From the lightest-colored fruit bodies of *P. duplex*, almost the only sign of color was the fact that it was possible to locate even a sparse spore print on the white paper, but we consider it such a sign, because equally sparse prints of quite colorless spores are not visible to us against a white background.

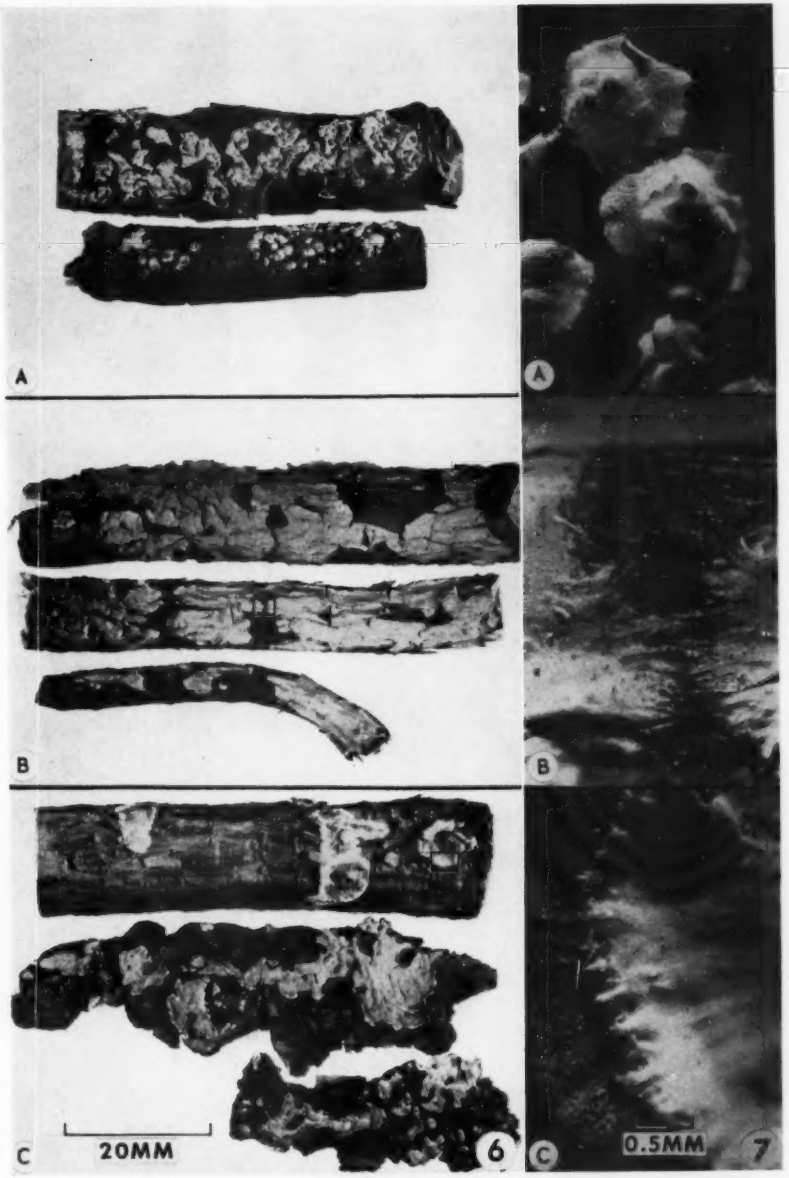
Sporocarp Color

Color is a variable character within each taxon of this group and, as Boidin ([1959], p. 439) has pointed out, difficult to match with Code colors when fresh; and we have found it little better when dry. A single collection, showing all stages of growth from youth to age, when dry may show a range of colors from a definite pink to a dusky purple or dark brown. Because almost any tone that occurs in one may occur in either of the other two taxa, it may be almost misleading to point out that, in young fruit bodies, dried, a predominating and concolorous cream or tan is likely to occur in *P. duplex*, whereas the pink will usually stand out more clearly in American and European *S. pini*, with the central region typically distinctly darker in American *S. pini*. In older dried fruit bodies, a more definite purple and a deeper brown are more likely to be found in the latter two, while *P. duplex* generally achieves only a greying cinnamon or a drab purple.

In color of the dried fruit body, therefore, *P. duplex* can often be distinguished from the other two.

FIG. 6. Macro-appearance of sporocarps. Fig. 7. Macro-appearance of margin. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*) from DAOM *53381*. B. *P. duplex* (*P. pini* subsp. *duplex*) from DAOM *31785*. C. American *S. pini* (*P. pseudo-pini*): 6-C from DAOM *30466-A* and *31795*; 7-C from DAOM *30466-A*.

PLATE I



Weresub and Gibson—Can. J. Botany

PLATE II

Weresub and Gibson—Can. J. Botany

Growth and Gross Structure (*Fig. 6*)

A group of orbicular fruit bodies may be found as the early stage of growth of any of these three fungi, but the pulvinate depth which American and European *S. pini* can attain is quite unknown in *P. duplex*. Furthermore, *P. duplex* rarely retains the individuality of its fruit bodies; its usual occurrence is in a more or less effused confluence of sporocarps (Fig. 6-B), so that their original singleness is frequently lost in a rather uniform unit. American *S. pini* (Fig. 6-C), on the other hand, even in confluence often maintains some sign of the individuality of the fruit bodies that have more or less united. And it would seem from the descriptive comments of European authors (e.g. Pilát 1930: "manchmal einige (aber niemals viele) nahestehende Fruchtkörper zusammenfliessend"), and what we have seen of European material (Fig. 6-A), that the growth of European *S. pini* is much like that of American *S. pini*.

Associated with the "shield shaped" (Burt 1920) individuality of the fruit bodies of American *S. pini* is the occurrence of a generally more pronounced central umbo in each. Some tuberculation may be seen in *P. duplex* as well, but rarely the clearly marked central core of what was originally a single fruit body, more frequently with apparently no relation to umbilical rooting. The same unrelated tuberculation may also occur in American *S. pini*, but it is there more likely to mark umbilical points. However, this may merge into an almost cerebriform convolution in both American and European *S. pini*.

Nobles, who has already pointed out (1956) that American *S. pini* causes a heart rot and *P. duplex* (her Unknown "T") a sap rot, has suggested to us that this manner of growth on the surface of the host may be determined by the location of the fungus within the host: since American *S. pini* travels from deep in a heart rot, it will emerge at few points, and its fruit bodies be relatively well developed before confluence takes place, whereas *P. duplex*, causing a sap rot close to the surface, emerges at many points simultaneously so that confluence is achieved before the individuality of many of the fruit bodies has been clearly established.

It seems that in gross structure, as well as in gross color, *P. duplex* usually differs from both American and European *S. pini*, which cannot be distinguished on those characters.

Margin (*Fig. 7*)

Special attention must be given to the margin of actively growing fructifications, since its appearance is often as clearly diagnostic a gross character as any to be found in this group.

The margin of American *S. pini* (Fig. 7-C), at its most characteristic, is perhaps the most striking: fimbriate and sometimes radiately fingered, typically set off as abruptly paler than the well differentiated parts of the fruit body,

FIG. 8. Micro-appearance of section of sporocarp. Fig. 9. Micro-appearance of section of margin. Fig. 10. Section of subiculum, more highly magnified. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*): 8-A, 9-A, 10-A-1 from DAOM *53381*; 10-A-2 from Czech. Union Dept. Agr. *40949*. B. *P. duplex* (*P. pini* subsp. *duplex*) from DAOM *31785*. C. American *S. pini* (*P. pseudo-pini*) from DAOM *31795*.

and often of so little depth that the fructification bulges behind it. Even when very young, the margin is frequently free to slightly raised, though portions may be somewhat adherent. As the fructification ceases to grow outward, the sterile margin becomes less and less marked, and the freeing of the subiculum from the substrate may proceed almost to the umbilical root; or the fruit body may remain more or less adnate, the margin (by this time with a differentiated hymenium) curling away from or toward the substrate.

The actively growing margin of *P. duplex* (Fig. 7-B) may be as fimbriate and occasionally almost as radiately fingered as in American *S. pini*, and is often pure white; but here the color of the fruit body typically pales only gradually outward, and the fructification is so shallow throughout that there is no abrupt drop from differentiated portion to sterile margin. Furthermore, this margin characteristically hugs the substrate, adhering at least basally, though upper fimbriations may lie free. And with age, when the sterile margin disappears, the edge of the fruit body may shell away from the substrate, but more typically remains appressed.

Our observations on the fructifications of European *S. pini* have been too few for a similar analysis of the appearance of its margin; but the comments of European authors (Secretan 1883: "les bords se détachent tant soit peu, sans jamais se replier"; Quélet 1888: "borde d'une marge fimbriée et crispée"; Herter 1910: "mit freiem, rundlich begrenztem, etwas abstehendem Rande"; Pilát 1930: "mit freiem Rande", "Rand erst striegelhaarig, weisslich bis weiss, dann kahl, scharf, gleichfarbig und von Substrat sich abhebend"; Skovsted 1956: "with free margin", "when young light brown with yellowish white margin"; Eriksson 1958: "loosening at the margins and at last fixed only in the centre"; and others) indicate an appearance, at least at maturity, that is closer to American *S. pini* than to *P. duplex*, whether or not these authors are all describing the same species.

Once again it seems possible to distinguish *P. duplex* from the other two, but not American *S. pini* from the European.

Texture

The differences in texture that are found in these three taxa seem to be associated with the depth of the fructification and the types of hyphae (cf. below) of which it is composed.

American *S. pini*, when well developed but not overmature, can usually be sectioned down to substrate with relative ease by the technique described above. This indicates a normally waxy to cartilaginous texture. Later, it may become tougher, and parts of the subiculum may approach a mealiness that is difficult to section.

European *S. pini* may be ceraceous or coriaceous throughout or, as in DAOM *31310* from Sweden, ceraceous in the hymenium and farinose elsewhere. It is often almost impossible to get a clean-cut section through a farinose specimen. When made, a section of its fruit body spreads in the mounting medium at the lightest tap on the cover slip, so that the organization of the

fructification is largely lost. In American *S. pini*, on the other hand, comparatively little spreading takes place, the entire mass of hyphae giving the appearance of immersion in a matrix, and considerable pressure and tapping is required before the relationship of subiculum to subhymenium is lost. In *P. duplex*, the fructification often sections well because of the ceraceous cohesiveness of all elements, also as if immersed in a matrix, but the papery thinness it may achieve may provide too little body for good sectioning by this technique.

On the basis of this character, American *S. pini* and *P. duplex* seem to be closer to each other than either is to European *S. pini*.

Microstructure of Section (*Figs. 8–10*)

In the shallow fruit body of *P. duplex* (Figs. 8-B, 10-B), almost any longitudinal even approximately radial section shows a good portion of clearly horizontal and parallel hyphae forming a distinct subiculum, usually uniform in depth, from as little as 25 μ to as much as 100 μ, only occasionally more. And the transition from horizontal hyphae to erect subhymenium is usually fairly abrupt.

American *S. pini* (Figs. 8-C, 10-C), too, has a generally well-marked horizontal subiculum with parallel hyphae, typically deeper; but it is usually apparent only in a definitely radial section, and the transition to the erect subhymenium is gradual, the area frequently as deep as the subiculum itself.

In the few collections we have seen of European *S. pini*, even what seems to be a perfectly radial section rarely shows a clearly marked parallelism in its more or less horizontal subiculum (Figs. 8-A, 10-A); and Pilát (1930), who gives the most detailed description of that taxon, says: "Trama . . . aus . . . dicht unregelmässig verwebten (nicht parallel angeordneten, wie bei der Mehrzahl der arten) . . . Hyphen bestehend". The most pronounced paralleling of subicular hyphae that we have seen in European *S. pini* is in the fructification of a Czechoslovakian collection No. *40949* (Fig. 10-A-2), but even here the hyphae are more closely interwoven than in American *S. pini* (Fig. 10-C).

The presence of a clearly paralleled horizontal subiculum, apparently embedded in a matrix, is characteristic of the American taxa of this group, and the difference in its depth can often (though not always) distinguish between American *S. pini* and *P. duplex*.

The definitely radial section of an actively growing margin may also be helpful in distinguishing American *S. pini* (Fig. 9-C) and *P. duplex* (Fig. 9-B). As mentioned above in the description of the gross margin, that of *P. duplex* is typically appressed at least basally, that of American *S. pini* usually raised throughout. In addition, it is worthwhile to note the development of sulphuric-benzaldehyde-positive gloeocystidia: these, in American *S. pini*, are frequently the first structures to be differentiated, sometimes present even in the otherwise completely undifferentiated marginal hyphae; and as differentiation into subiculum, transition area, and potential hymenium become observable, gloeocystidia will often become apparent in the transition and

subicular regions before or simultaneously with their occurrence in the potential hymenium. In *P. duplex*, on the other hand, their first appearance is most typically in the developing hymenium.

The illustrated marginal section from the fructification of European *S. pini* (Fig. 9-A) is not comparable to the margins shown in Figs. 9-B and -C, since it is of an already differentiated margin. In the collections of European *S. pini* at our disposal, only the Czechoslovakian collection already mentioned shows an undifferentiated margin. Since it may or may not be typical, we have not used it for illustration. Fig. 9-A does, however, show a typical view of the mature margin of even a small fructification of European *S. pini*.

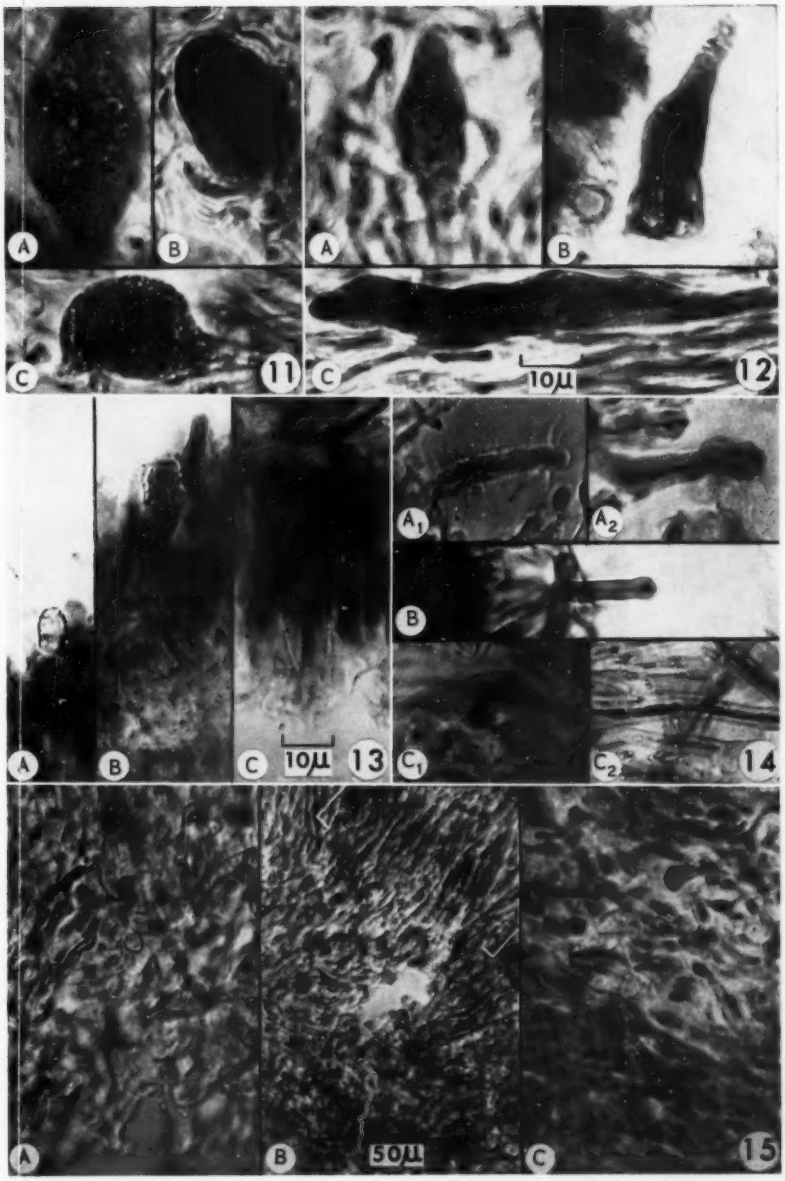
Hyphae (*Figs. 14, 15, 18, 19*)

Although Nobles (1956, p. 118) distinguished between American *S. pini* and the other species she covered in that paper by "the presence of hyphae with thick, gelatinously modified walls", and others have described gelatinous walls in hyphae of American *S. pini* (Burt 1920; Overholts 1939; and, as *Phlebia cervina*, Overholts 1930), of European *S. pini* (Bourdot and Galzin 1921 and [1928]; Pilát 1930; Skovsted 1956) and of *P. duplex* (Burt [1926]*a*; and as *Corticium overholtsii*, Burt 1926*b*, Overholts 1929*b*), only Eriksson has considered this gelatinization so strikingly different from wall-modifications in other members of the Coloratae that it warranted use as a generic character for segregation of *S. pini* and *S. rufum*. Confident of the scrupulousness of Eriksson's observations, we therefore scrutinized the hyphae of the three taxa with the hyphae of *S. rufum* in mind. And we found that Eriksson had indeed put his finger on a wall-differentiation that is different from that which occurs in other species of Coloratae: a swelling between outer and inner membranes of the wall, with or without a scaling away of the outer membrane.

In *P. duplex* (Figs. 14-B, 19-B), the hyphal wall may be barely measurable (Fig. 19-B-2, -4, -6), but it is more typically emphasized (Fig. 19-B-1, -3) to about 1 μ wide (Fig. 19-B-7), and in the subiculum may swell to a width of 2.5 μ (Fig. 14-B, 19-B-5), so that the lumen may be capillary, the hyphal diameter being fairly uniform, usually ranging between 3 and 5 μ. Occasionally a few basal hyphae show signs of the type of erosion illustrated for European *S. pini* (Fig. 14-A-2), or a differential dissolution that leaves the hyphae somewhat rough-walled to markedly cross-striate (Figs. 14-A-1, 19-A-2, -5, -8) or "zippered" (a term for which we are indebted to Dr. Nobles). But in *P. duplex*, these hyphae are rare.

In American *S. pini*, subicular hyphae (Figs. 14-C, 19-C-5 to -9) are generally from 4 to 7 μ in diameter; the swelling of the hyaline wall may bring it to a width of 2.5 μ, the lumen appearing capillary or not (depending on the diameter

FIGS. 11–15. Microstructures from sporocarps. Fig. 11. Vesiculose gloeocystidia. Fig. 12. Elongate gloeocystidia. Fig. 13. Cystidia. Fig. 14. Subicular hyphae. Fig. 15. Brown-walled hyphae and hyphal tips. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*): in 11 to 14 from DAOM *53381*; in 15 from Lundell collection, 4.V.33, at TRTC. B. *P. duplex* (*P. pini* subsp. *duplex*): in 11, 13, and 15 from DAOM *53384*; in 12 from DAOM *31788*; in 14 from DAOM *31785*. C. American *S. pini* (*P. pseudo-pini*): in 11 and 12 from DAOM *31795*; in 13, 14-C-1, and 15 from DAOM *30521*: in 14-C-2 from DAOM *30466-A*. Scale in Fig. 12 applies also to Fig. 11; scale in Fig. 13 applies also to Fig. 14.


A
B
C
11
A
B
C
10μ
12
A
B
C
10μ
13
A1
A2
B
C1
C2
14
A
B
50μ
C
15

of the hypha itself), sometimes irregular in outline (Fig. 19-C-8). In this taxon too, erosion and zippering may occur, perhaps less rarely than in *P. duplex*, but still not commonly, and also confined to basal hyphae.

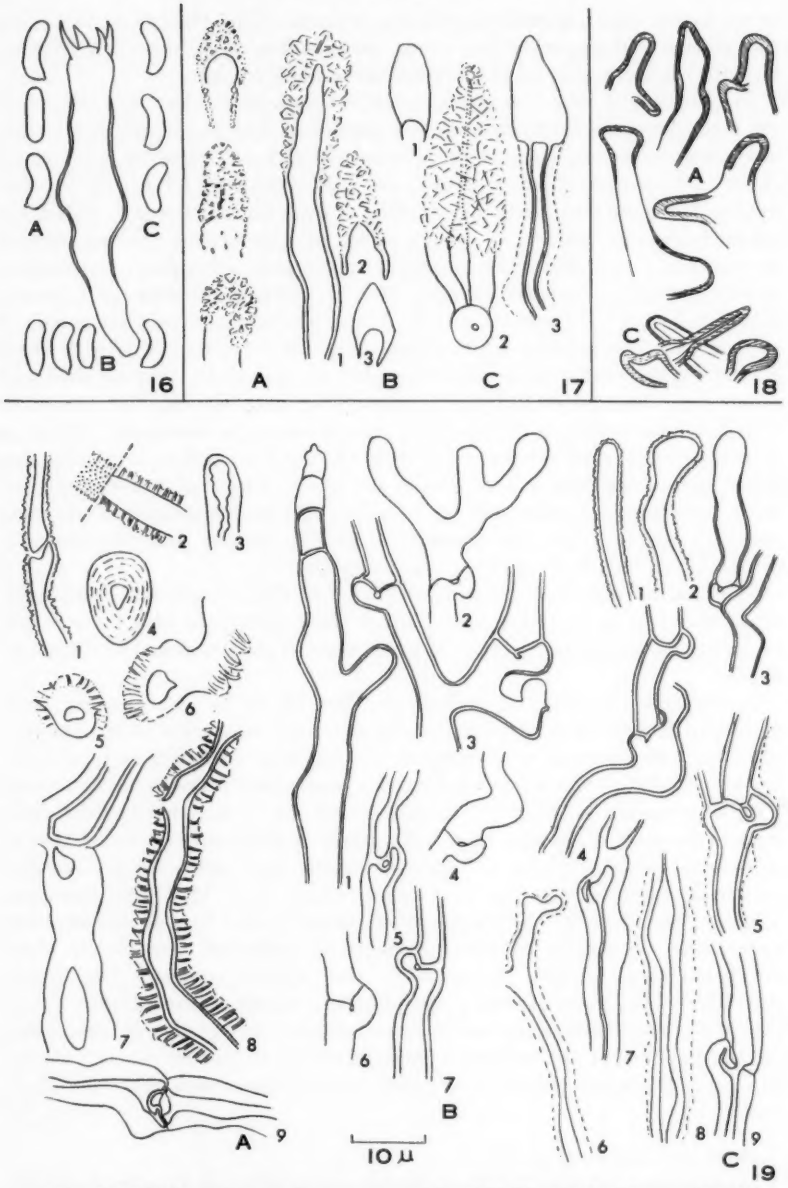
In European *S. pini*, the swollen walls of the hyphae (Figs. 14-A, 19-A-4 to -9) throughout the subhymenium and subiculum leap to the eye in a freshly made mount, the hyphae sometimes as narrow as 5 μ in diameter, but usually closer to 10 μ wide when uneroded, the wall as little as 1.7 μ wide in some hyphae but more frequently approaching or even exceeding 4 μ in width, the lumen frequently uneven, as though pulled and pressed by various stresses. In fructifications of this taxon, erosion of hyphal walls is frequent, the diameter of a hypha variable because of it (Fig. 14-A-2); and in some cases the zippering shown in Figs. 14-A-1 and 19-A-2, -5, -6, and -8 is so abundant that masses of hyphae look peppered in surface view (Fig. 19-A-2). And it is with these greatly swollen walls, whose outer membranes apparently may be shed and which may become eroded and cross-striate, that the friable texture of fructifications, mainly of European *S. pini*, seems to be associated. Again, as in the paralleling of the subicular hyphae, the Czechoslovakian collection *40949* approaches American *S. pini* in the rarity of such wall-differentiation. But other European collections we have seen can be distinguished from most American specimens by the presence of swollen hyphae well into the subhymenium as well as throughout the subiculum.

Marginal hyphae (Figs. 19-A-1, -3; -B-1, -2; -C-1, -2) are not characteristically different in the three taxa. Any of those illustrated may occur in all, though the rough-walled hyphae may be more frequently found in American *S. pini*.

Brown-walled hyphae and hyphal tips (Figs. 15, 18) are, on the other hand, significant in distinction of these taxa. Brown zones may occur in all, from the mid-hymenium down to subhymenium or subiculum. In *P. duplex* (Fig. 15-B: bracketed area), it is usually a matter of a generalized yellowing or browning, a resinous deposit between the hyphae which only sometimes engrains their walls to the point of coloring them individually or penetrates the outer layer to present the appearance of a thickened brown wall, either on an elongate portion of a hypha or its tip. In American (Figs. 15-C, 18-C) and European *S. pini* (Figs. 15-A, 18-A), however, these brown-walled hyphae and browned hyphal tips, branched, swollen, attenuate or contorted, are clearly characteristic, when present. On occasion, they almost approach the loosest branching in the *P. versiformis* group. The appearance of these brown-walled hyphae against a background of hyaline structures can be taken as eliminating the possibility that the collection under study is *P. duplex*. Unfortunately, they are not always present in *S. pini*, and are sometimes mimicked in *P. duplex*.

Gloeocystidia (*Figs. 11, 12, 20*)

These occur in greater or lesser abundance in all three taxa, more or less strongly positive in sulphuric-benzaldehyde, and variable in shape and size. Any of those illustrated, and other variations of them, may occur in all three.



FIGS. 16–19. Microstructures from sporocarps (*cont'd.*). Fig. 16. Basidium and basidiospores. Fig. 17. Cystidia. Fig. 18. Brown-walled hyphal tips. Fig. 19 Hyphae. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*) from DAOM *53381*. B. *P. duplex* (*P. pini* subsp. *duplex*) from **type**. C. American *S. pini* (*P. pseudo-pini*): in 16, 17-C-1 and -C-3, 19-C-3 to -8, from **type**; in 17-C-2, 18-C, 19-C-9, from DAOM *30124*; in 19-C-1 and -2 from DAOM *31795*.

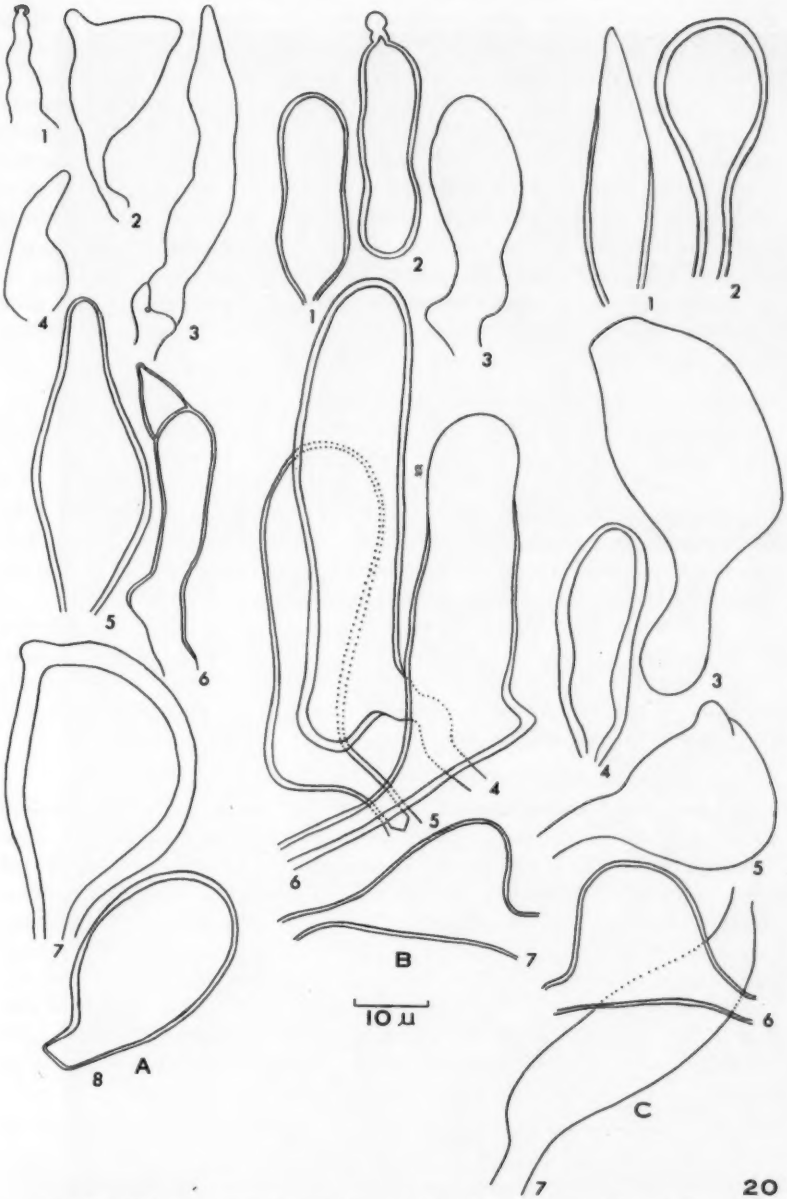


FIG. 20. Microstructures from sporocarps (*concl'd.*): Gloeocystidia. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*): A-1 to -3, -5, and -8, from DAOM *53381*; A-4, -6, and -7, from DAOM *53382*. B. *P. duplex* (*P. pini* subsp. *duplex*) from **type**. C. American *S. pini* (*P. pseudo-pini*): C-1 from DAOM *30124*; C-2 to -7 from **type**.

However, the elongate gloeocystidium that is somewhat conical in shape (Figs. 12-B, 20-B-6) is typical of *P. duplex*, though it may occur in the other two. And the vesiculose gloeocystidium (Figs. 11; 20-A-7, -8; -B-7; -C-3, -5, -6) is typical of American and European *S. pini*, though it may also appear in *P. duplex*. The vesicle, when properly delimited, may be used as a diagnostic character: only American and European *S. pini* show a consistent, sometimes abundant occurrence of gloeocystidia whose swollen portion (exclusive of whatever stalk or rostrum may be present) measures less than two to one in relation of length to width, and more than 12 μ in diameter. In American *S. pini*, the presence of numerous brown-walled hyphae sometimes seems to act as a substitute for vesicles, but usually younger fruit bodies in the same collection will yield sufficient evidence of the prevalence of vesiculose gloeocystidia.

Cystidia (*Figs. 13, 17*)

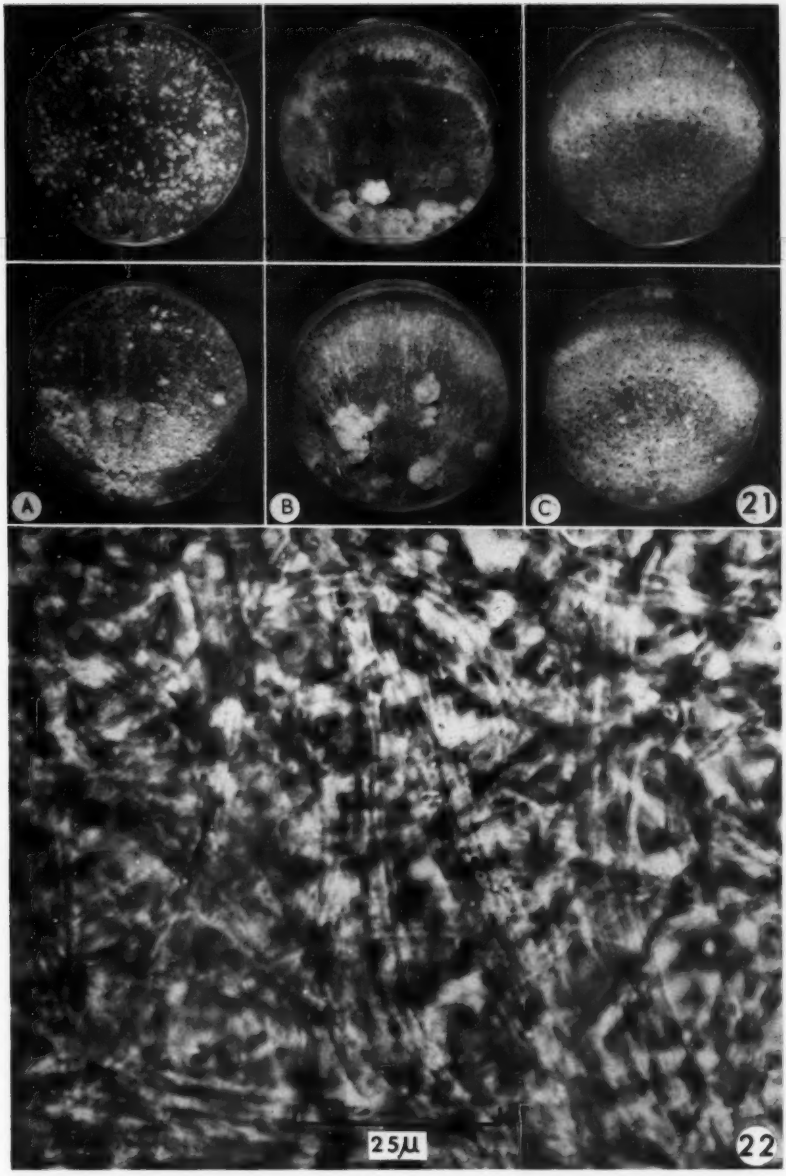
Cystidia with heavily thickened walls below and coarse crystal-covered apices as much as, say, 60 × 15 μ in size (Figs. 13-C, 17-C-2) are to be found only in American *S. pini*; bases with somewhat thickened walls and apices that measure as much as 35 × 14 μ occur in *P. duplex* (Figs. 13-B, 17-B-1); while in European *S. pini*, only the small cystidium (Figs. 13-A, 17-A), with thin basal walls and crystal-covered apex no more than about 20 × 7 μ has been seen. However, the range is such that any of the cystidia illustrated may have been drawn from American *S. pini*, from which the characteristic large cystidium is frequently absent; all except Fig. 17-C-2 might have come from *P. duplex*; whereas Figs. 13-C, 17-B-1, -C-2, and -3 could not be found in European *S. pini*. In all, the numbers of cystidia may vary from too few to find to too numerous to miss.

Basidia and Basidiospores (*Fig. 16*)

Only one basidium has been drawn, since it is representative of the type occurring in all three taxa: long, flexuous, subclavate, with or without some slight thickening of the wall where the basidium is widest. It varies in length and in width of apex as well as of mid-point, but seems to show no significant differences between taxa in this group. The basidiospores present what appears to be a statistical rather than an individual difference in size between the taxa: in European *S. pini*, usually the upper range in length (7–8.5 μ) and the middle range in width (2–2.8 μ), so that the spore is comparatively slender (Fig. 16-A); in American *S. pini*, generally a shorter spore (6–7.5 μ), also slender (2.2 μ) (Fig. 16-C); and in *P. duplex*, a broader spore in relation to its length (Fig. 16-B), usually 5.5–7.5 × 2.2–2.8 μ.

FIG. 21. Gross appearance of polysporous cultures on malt agar: top row, 4 weeks' growth; bottom row, 6 weeks' growth. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*) from DAOM *53381*. B. *P. duplex* (*P. pini* subsp. *duplex*) from DAOM *31786*. C. American *S. pini* (*P. pseudo-pini*) from DAOM *31793*. FIG. 22. Mesh-like network of hyphal strands in culture of *P. duplex*.

PLATE IV



Cultural Characters: Observations and Comparisons

Gross Appearance of Non-fruiting Cultures (Fig. 21)

In the early stages of growth, polysporous isolates of these three taxa look much alike. The mycelium is thin and subfelty, or slightly raised, woolly floccose. Then noticeable differences appear in about two weeks.

In American *S. pini*, minute droplets of brown, resinous material are found clinging to the hyphae during active growth. Though not all isolates of this taxon show these droplets, their occurrence may be considered characteristic of American *S. pini*, for neither *P. duplex* nor European *S. pini* is known to produce them, though patches or spots of brown may develop in all three.

As growth continues, European *S. pini* takes on a mealy or farinose appearance due to minute flecks of compact mycelium dotted over the mat. Similar flecking may occur sparsely in American *S. pini*, but here, whatever mycelium remains aerial as the culture develops becomes loose cottony (Fig. 21-C). In European *S. pini* (Fig. 21-A) and in *P. duplex* (Fig. 21-B), however, much of the aerial mycelium typically becomes concentrated into raised tufts or clumps or balls of hyphae, varying in size, distribution, and number, usually large and prominent in *P. duplex*, apparently smaller in European *S. pini*. It must be emphasized at this point that only two isolates of the latter have been studied thoroughly: we have, therefore, no information on its potential variability.

American *S. pini* differs from the other two taxa also in always producing some brown coloring within the agar, so that the reverse is characteristically colored. The color produced by *P. duplex* and European *S. pini* seems to be largely in the mycelium, the reverse remaining unchanged or, in *P. duplex*, becoming dotted or patched with brown.

An almost infallible diagnostic character, when it is present, is the "dried coconut" odor referred to by Nobles (1956) as occurring in the cultures of most isolates of American *S. pini*. It will remain for future studies to reveal the possible significance of the faint suggestion of spearmint odor we have noted in cultures of European *S. pini*. Those of *P. duplex* are consistently odorless.

From this, it appears possible, with a large number of isolates, to separate cultures of American *S. pini* from the other two taxa on the basis of these differences in gross appearance and odor. But so far, European *S. pini* and *P. duplex* seem to be inseparable.

Hyphae (Figs. 22, 23)

The first character to be observed when examination is made of a crushed mount of the mycelium taken from a culture of *P. duplex* is the arrangement of the hyphae. By about 10 days after inoculation, the individual hyphae consistently become aggregated into strands that branch and anastomose to form a mesh-like arrangement (Figs. 22; 23-B-3). This meshwork is also found, but is less pronounced, in European *S. pini*, but it is not known in American *S. pini*: another cultural character that supports our genetic evidence.

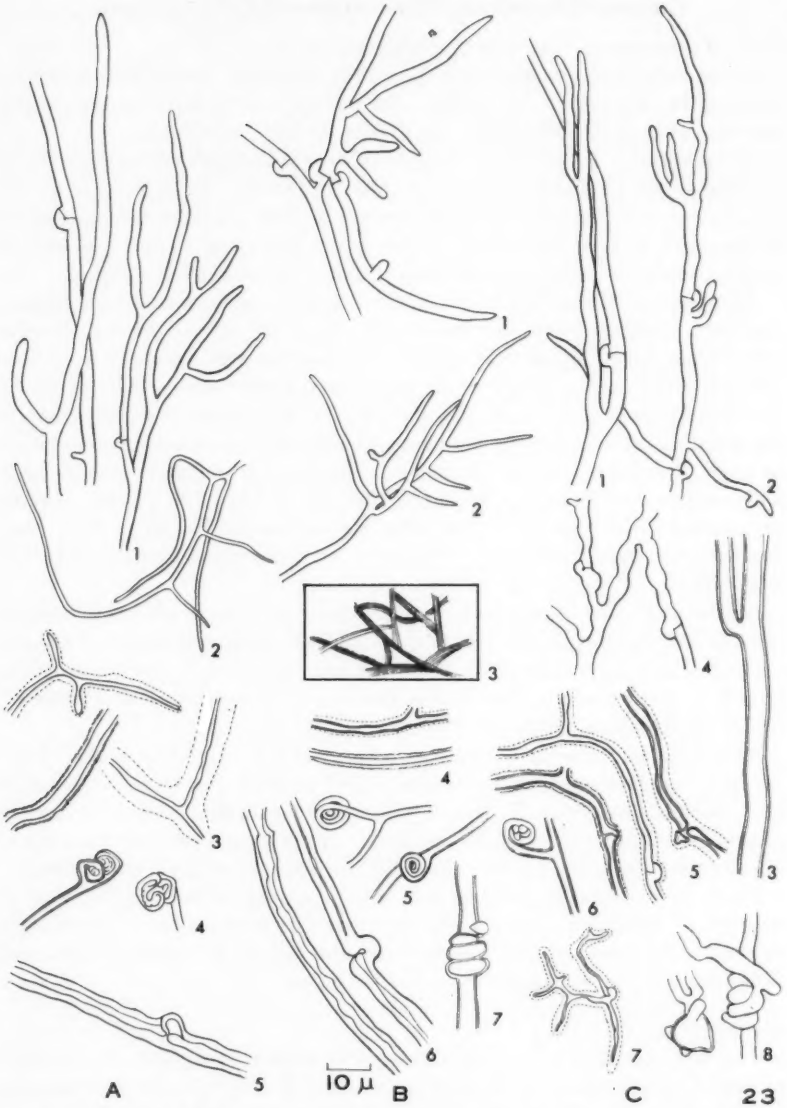


FIG. 23. Mycelium from cultures. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*): A-1 from DAOM *53381* and *53382*; A-2 to -5 from DAOM *53381*. B. *P. duplex* (*P. pini* subsp. *duplex*): B-1 from DAOM *31790*; B-2 from DAOM *53383*; B-4, -6, and -7, from DAOM *31784*; B-5 from DAOM *53384*; B-3, rough sketch of hyphal network photographed in Fig. 22. C. American *S. pini* (*P. pseudo-pini*): C-1 to -3 from DAOM *31218*; C-4 from DAOM *31214*; C-5 and -8 from DAOM *31793*; C-6 from DAOM *21017*; C-7 from DAOM *30124*.

When young, actively growing cultures, not yet covering the petri plate, are examined microscopically, many of the aerial hyphae in all three taxa are seen to end in a cluster of short branches. And it is found that, though branches of the same type may occur in all three, there is a type characteristic of, but not invariably found in *P. duplex* and European *S. pini*. These branches (Figs. 23-A-2, -B-2) are very slender (as narrow as 0.8 μ in diameter), tapering gradually to a fine, needle-like point, and produced so profusely in their clusters that details of individual hyphae may be difficult to distinguish. The other type of branch that is found in clusters on the aerial hyphae of young cultures of all three taxa, and illustrated for American *S. pini* (Fig. 23-C-2), is not so narrow (not less than 1.6 μ diameter) and is more blunt, though it may taper at the extreme tip. The clusters of very fine branches may, therefore, be of diagnostic assistance, their presence in a culture ruling out the possibility that it is an isolate of American *S. pini*; but their absence is of no diagnostic value.

Gloeocystidia (*Fig. 24*)

What we are accustomed to calling gloeocystidia in cultures (i.e. structures with oily to granular or opaque contents) appear in non-fruiting cultures of these taxa in varying shapes and numbers.

American *S. pini* (Fig. 24-C) is characterized by the consistent presence of gloeocystidia of hyphal diameter (Fig. 24-C-1), that is, ends of hyphae distinguished from normal hyphae only by their gloeocystidiate contents. These are present throughout the non-fruiting mat, along with somewhat broader, also hyphal, gloeocystidia (Fig. 24-C-2, -4), as well as the occasional irregular to globoid structure (Fig. 24-C-3, -5). *P. duplex* does not always produce gloeocystidia, and when it does (Fig. 24-B), they are typically broader than the diameter of a hypha, and are more usually found in the tufts where the culture may be preparing to fruit than in the appressed mycelium. European *S. pini* (Fig. 24-A) resembles American *S. pini* in that gloeocystidia occur throughout the mycelium more generally than in *P. duplex*, and that those of hyphal diameter may be produced as well as gloeocystidia of broader diameter and irregular shape, though the former more rarely than in American *S. pini*. But the European taxon differs from the two American taxa in that its gloeocystidia that are produced in the appressed mycelium may be either negative or positive in sulphuric-benzaldehyde, whereas in *P. duplex* only an occasional gloeocystidium, and that only from a tuft, reacts positively, while there is no reaction to this reagent in non-fruiting cultures of American *S. pini*.

Using gloeocystidia alone, we may have characteristics on which to separate the three taxa involved here: the presence of numerous hyphal gloeocystidia, accompanied or not by other types, and none sulphuric-benzaldehyde-positive, points to American *S. pini*; the presence, in the appressed mycelium, of at least some gloeocystidia that are positive in sulphuric-benzaldehyde, suggests European *S. pini*; and the rarity of gloeocystidia anywhere except in the tufts of mycelium indicates *P. duplex*.

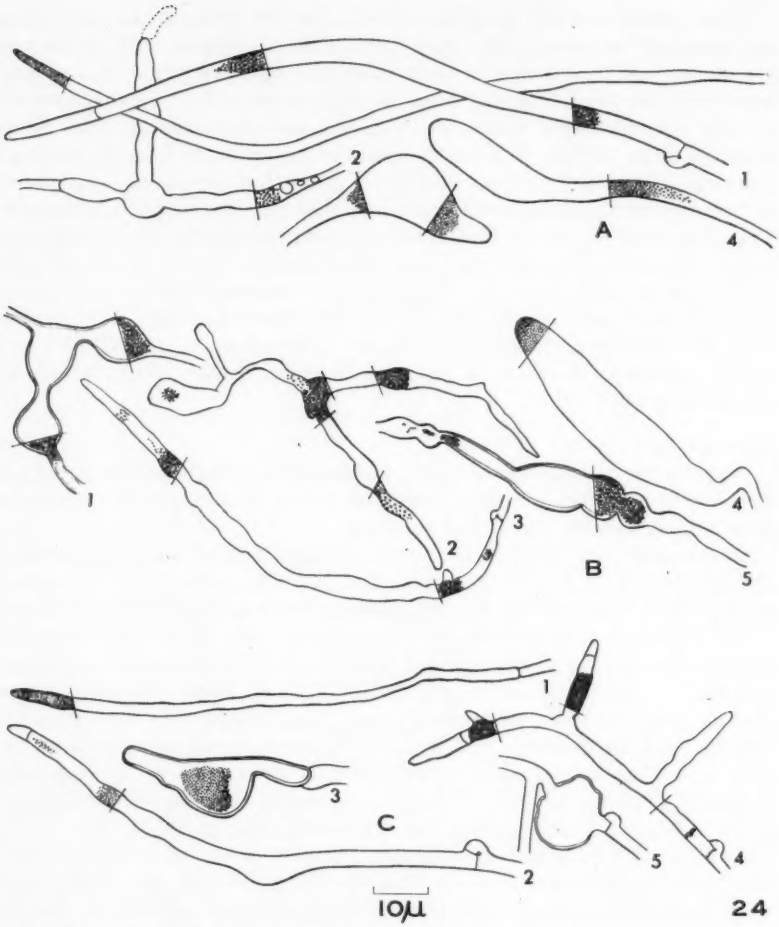


FIG. 24. Gloeocystidia from cultures. A. European *Stereum pini* (*Peniophora pini* subsp. *pini*) from DAOM *53381* and *53382*. B. *P. duplex* (*P. pini* subsp. *duplex*): B-1 and -2 from DAOM *31785*; B-3 to -5 from DAOM *31792*. C. American *S. pini* (*P. pseudo-pini*): C-1 from DAOM *31215*; C-2 from DAOM *31214*; C-3 from DAOM *31793*; C-4 and -5 from DAOM *21017*.

Conclusions

This rather lengthy discussion has been an attempt to show that we are dealing here with three taxa that are so close in morphology that all characters overlap, and yet whose fruit bodies in nature are identifiable as one or other of the three, and whose cultures can be divided at least into the two groups that coincide with the intersterile and partially intrafertile groups of our interfertility studies.

American *S. pini* is different: it does not mate with either European *S. pini* or *P. duplex*; it is morphologically distinguishable both in culture and in nature. To maintain it as conspecific with European *S. pini* would be to ignore both genetic and morphological evidence. And yet, to suggest that its relationship to *P. duplex* and European *S. pini* is no closer than to other species of the same genus is to disregard the numerous similarities in structure among the three.

P. duplex and European *S. pini* are, as far as we have been able to judge, amply distinct in nature, but barely separable in non-fruiting culture. Furthermore, they have shown clear evidence of a capacity to unite their hyphae in a dicaryon mycelium. They are certainly more closely related to each other than either is to American *S. pini*. Rogers and Jackson's (1943) suggestion that *P. duplex* might be "a growth phase or variety of *S. pini*" has been corroborated, though not quite as they anticipated.

This we can say in spite of our obvious ignorance of the circumscription of the European taxon, and in spite of the possibility that more than one such taxon occurs in Eurasia, or that "European *S. pini*" occurs in North America. Boidin's promised paper on the species in Europe may solve both of the first two questions, and further study of additional collections here will help settle the third. Meanwhile we feel that our limited data do provide some clarification of the problem as we know it today.

Since our classification should reflect both the closeness in morphology of these three and the genetic and geographic barriers between them, this seems to us to be the logical place for the use of the "aggregate species", as the term has been used by, for example, Valentine and Löve (1958) and Manton (1958): *S. pini* aggregate is a distinct morphological grouping, within which a finer delimitation is possible. On the basis of both morphology and intersterility, American *S. pini* becomes a separate species. Because of the retention of some capacity for interfertility, *P. duplex* and European *S. pini* are together regarded as a second species, but their morphological differences and geographic disjunction require their classification as separate subspecies.

Finally, a decision must be made regarding the genus. As has already been mentioned here and elsewhere by many authors, *S. rufum* and *S. pini* are not related to the genus *Stereum* as typified by *S. hirsutum* but they are closely related to each other and to the genus *Peniophora* s. str. Eriksson has separated these two species from the genus *Peniophora* on the basis of gelatinized hyphae and colorless spore print. To Boidin (1956 etc.) and to us, the spore prints of these two taxa deviate as much from the colorless as do the prints of some of the other species of the genus. The hyphae are different from those in most members of the Coloratae, but the difference hardly seems to us to be of generic consequence when so many other characters tie these species to the group (cf. Nobles 1956, p. 104: "The cultural characters of [American] *Stereum pini* . . . , *Peniophora septentrionalis* Laurila, and an unidentified species occurring on pine [*P. duplex*] are so similar as to make identification extremely

difficult"). What Eriksson would choose to treat as a suprageneric link (*Sterellum* as a 'satellite' genus to *Peniophora*) we prefer to regard as a subgeneric bond.

As we know it at the monent, the subgenus includes *P. pini* aggr., i.e. *P. pini* (Schleich. ex Fr.) Boid. subsp. *pini*, *P. pini* subsp. *duplex* (Burt) stat. nov., and *P. pseudo-pini* nom. nov. (for American *S. pini*, i.e. *Phlebia cervina* Overh., non *Peniophora cervina* (Thüm.) Höhn. & Litsch.); and *P. rufa* (Fr.) Boid. It might therefore be called the subgenus *Sterellum* (Karst.). However, it is possible that *P. quercina*, *P. suecica*, and *P. septentrionalis* may find their place in the same subgenus, which would then automatically be referred to as subgenus *Peniophora* because it included *P. quercina*, type species of the genus. Until such time as a thorough study of these other species clarifies this point, we shall leave the subgenus unnamed.

Key to Fruit Bodies of the Species of *Peniophora* s. str.

(based on Eriksson 1950; Nobles 1956; Boidin 1958*b*, and [1959]; and present study)

1. Basidiospores ellipsoid, ratio of length to width equal to, or less than, 2.5 to 1 ..... *P. lilacea* group, *P. incarnata* gp.
1. Basidiospores cylindrical, ratio of length to width equal to, or greater than, 2.5 to 1 2
   2. Consistently with dendrohyphidia, hyaline or brown, entering hymenium or at least subhymenium ..... *P. lycii* gp., *P. polygonia* gp., *P. versiformis* gp.
   2. Lacking true dendrohyphidia, though possibly possessing some brown-walled hyphae with a sparse dichotomy of tips 3
3. Distinct basal layer lacking in fruit body or, if present, composed wholly of brown-walled, densely cemented hyphae ..... *P. cinerea*, *P. junipericola*, *P. limitata*, *P. nuda*, *P. piceae*, *P. pithya*, *P. rufo-marginata*, *P. separans*, *P. violaceo-livida*
3. Distinct basal layer present in fruit body, usually relatively horizontal, largely unpigmented except for brown zones; subicular hyphae relatively loose, though possibly appearing as if embedded in a hyaline matrix 4
   4. Considered restricted to wood of deciduous trees ..... *P. quercina*, *P. rufa*, *P. suecica*
   4. Considered restricted to wood of coniferous trees 5
5. Gloeocystidia (usually sulphuric-benzaldehyde-positive) elongate, typically equal to or exceeding 7 to 1 in ratio of length to width, regularly arranged in hymenium but originating well below it; always accompanied, in hymenium just behind margin, by emergent cystidia with coarsely encrusted apices 7.5 μ wide and with basal walls heavily thickened ..... *P. septentrionalis*
5. Gloeocystidia broader in relation to their length, rarely approaching or exceeding 7 to 1 in ratio of length to width; if such slender gloeocystidia present, cystidia in young hymenium less coarsely encrusted, narrower, and with basal walls little thickened ..... *P. pini* aggr. 6
   6. Individual brown-walled hyphae traversing hyaline areas of subiculum or subhymenium, with brown-walled tips (swollen, contracted, or dichotomously branched) in subhymenium or entering hymenium 7
   6. Such hyphae and tips, if at all present, associated only with browned zone or area 8
7. Radial section with distinct subiculum of parallel horizontal hyphae; these frequently appearing as if embedded in a matrix, their diameter rarely exceeding 7 μ, with walls only slightly thickened or swollen to as much as 3 μ in width, usually smooth, sometimes eroded, or cross-striate or roughened ..... *P. pseudo-pini*
7. Radial section with distinct subiculum, but of mainly interwoven rather than parallel hyphae; these separable, brittle, in diameter frequently exceeding 7 μ, with walls typically greatly swollen, sometimes to as much as 4.5 μ in width, often uneven in outline, eroded or cross-striate ..... *P. pini* subsp. *pini*
   8. In distinctly radial sections, vesiculose gloeocystidia (2 to 1 in ratio of length of swollen part to its width, width exceeding 12 μ) rare in any one mount, totally absent from most such mounts ..... *P. pini* subsp. *duplex*
   8. Vesiculose gloeocystidia (as above) numerous to few but consistently present in almost all such mounts (except perhaps in fruit bodies where brown-walled hyphae predominate) 7

Key to Non-fruiting Cultures of Conifer-rotting Species of *Peniophora* s.str.

(based on information from Nobles 1956, and present study)

1. Mycelium composed of hyphae with thin or slightly thickened walls, none surrounded by a gelatinous sheath 2
1. Mycelium composed of hyphae with thin or thickened walls, among them some surrounded by a gelatinous sheath ..... *P. pini* aggr. 4
2. Hyphae simple-septate, except for rare single clamp connections in the advancing zone ..... *P. separans*
2. Hyphae nodose-septate 3
3. Gloeocystidia numerous; prong-like branches on narrow hyphae in newer growth, tips coiling to form mass in which hyphal walls indistinct; odor lacking or slight, sweet ..... *P. septentrionalis*
3. Gloeocystidia present only in cultures at least 6 weeks old; prong-like branches lacking; strong iodoform odor ..... *P. pithya*
4. Mat lacking raised cottony tufts; mycelium frequently flecked with clinging brown resinous droplets; reverse browned; meshwork of hyphae lacking; gloeocystidia sulphuric-benzaldehyde-negative, those of hyphal diameter always present; odor of dried coconut usually distinct ..... *P. pseudo-pini*
4. Mat more or less pronouncedly tufted; droplets of brown resinous material lacking; reverse unchanged or brown-spotted or -patched; hyphae aggregated into more or less pronounced strands forming meshwork; gloeocystidia negative or positive in sulphuric-benzaldehyde, those of hyphal diameter rare or absent; odor lacking or faintly spearmint-like ..... *P. pini* 5
5. Hyphal meshwork present but not pronounced; gloeocystidia rare to numerous throughout, sulphuric-benzaldehyde-negative or -positive wherever found; odor sometimes faintly spearmint-like ..... *P. pini* subsp. *pini*
5. Hyphal meshwork distinct; gloeocystidia lacking or rare, more frequently found in tufts, usually negative in sulphuric-benzaldehyde, occasionally positive in tufts; odor lacking ..... *P. pini* subsp. *duplex*

Descriptions

Peniophora pini aggr.

SPOROCARPS

FRUIT BODIES (Fig. 6) discretely orbicular to confluent to effused; smooth or distinctly umbonate at one or several points to cerebrally convolute, flat, or raised or reflexed in age. COLOR variable, from pale cream or clay or flesh to tan, rosy brown or dark brown, to dusky or brownish purple; concolorous throughout or distinctly darker centrally, especially at umbos, edge abruptly or only gradually lighter. Actively growing MARGIN (Fig. 7) fimbriate, frequently white, appressed or slightly raised; mature margin fingered or entire, appressed or free. SURFACE usually smooth pruinose. TEXTURE papery or ceraceous or indurated or tough-coriaceous.

In SECTION, entirely colorless or browned in zones (*a*) in the basal part of the subiculum or (*b*) from the transition area halfway or fully into the hymenium or in patches throughout the context, browning due either to a deposit between hyphae or an impregnation of the walls or cytoplasmic content of the hyphae. CONTEXT (Figs. 8–10) composed of a subiculum of more or less horizontal, parallel or intertwined hyphae, a transition area of oblique hyphae turning erect, and an erect subhymenium and hymenium; depth (less hymenium) 50–400 μ. Apparent matrix present or absent.

HYPHAE (Figs. 14, 19) 3–11 μ in diam., with wall thin, emphasized (i.e. up to 1 μ in diam.) or thickened to as much as 4.5 μ; clearly nodose-septate throughout. Most hyphae with hyaline content and walls, except in darker-colored areas or fructifications where brown-walled or resin-filled hyphae predominate. Brown-walled hyphae (Figs. 15, 18) sometimes giving rise directly to cystidia or gloeocystidia, or ending in the subhymenium or hymenium in swollen, attenuate, or contorted tips, occasionally branching dichotomously some distance from a septum.

GLOEOCYSTIDIA (Figs. 11, 12, 20) varying from rare to very abundant; weakly to, usually, strongly blue-black in sulphuric-benzaldehyde, some browning or filled with brownish resinous content that does not react to this reagent (in dried specimens); observed throughout, from subiculum to hymenium; very variable in shape, whether newly formed (Fig. 20-A-1 to -4, -B-3) or mature: hyphal, elongate flexuous (Fig. 12-C), subfusoid (Figs. 12-A; 20-A-5, -C-1, -4), subcylindrical (Fig. 20-A-6, -B-1, -2, -4, -5), subconical (Figs. 12-B; 20-B-6), irregularly or one-sidely spindle-shaped (Fig. 20-B-7,-C-6, -7), or strongly vesiculose to globoid, with or without stalk or rostrum; variable also in size, in length from 12 to 100 μ, in width from 5 to 27 μ; wall thin or thickened, sometimes swollen to as much as 3.5 μ, usually colorless but sometimes yellowed or browned.

CYSTIDIA (Figs. 13, 17) rare to abundant, projecting or embedded, sometimes deep in subiculum; when young, thin-walled, typically subfusiform or subconical in shape (not positive in sulphuric-benzaldehyde); when mature, wall of stalk thin (Figs. 13-A; 17-A), emphasized (Figs. 13-B; 17-B-1), or thickened (Figs. 13-C; 17-C-2, -3), apex coarsely encrusted or lightly scattered with crystals; total length from 15 to 60 μ; crystalline apex 7–50 μ in length, 5-15 μ in diameter. Crystalline masses sometimes scattered along longer or broader structures in the subiculum and subhymenium, but not as cystidial apices.

BASIDIA (Fig. 16-B) long, flexuous-clavate, ca. 4.5–7 μ broad at the apex, with four slender, arched sterigmato. BASIDIOSPORES (Fig. 16) cylindrical, straight to almost reniform, 5.0–)6–8.5(–9) × (1.7–)2–2.8(–3.3) μ.

HOSTS

Pinus spp. (rarely other conifers); usually attached dead branches or dead saplings.

DISTRIBUTION

Reported throughout Europe and into Siberia and Mongolia (Secretan, Myc. Suisse **3**: 199. 1833; Streinz, Nomencl. fung. p. 605. 1862; Fries, Hym. Europ. p. 643. 1874; Karsten, Myc. Fenn. **3**: 309. 1876; Rabenhorst, Krypt. Fl. **1** (1): 341. 1884; Quélet, Ench. Fung. p. 206. 1886, and Fl. Myc. Fr. p. 13. 1888; Saccardo, Syll. Fung. **6**: 574. 1888; Britzelmayr, Hym. Sudbayern **10**: 220. 1894; Bresadola, Atti Accad. Sc. Lett. Arti Ag. Revereto III, **3**: 107. 1897, and Ann. Myc. **1**: 92. 1903; Höhnel & Litschauer, Wiesner-Festschr. p. 59. 1908; Smith, Syn. Br. Basids. p. 406. 1908; Herter, Pilze Krypt. Mark Brand. **6** (1): 126. 1910; Oudemans, En. Syst. Fung. **1**. 1919; Bourdot & Galzin, Bull. Soc. Myc. Fr. **37**: 120. 1921, and Hym. de Fr. p. 379. [1928]; Rea, Brit. Basids. p. 666. 1922; Pilát, Mykologia **3**: p. 6 (of reprint). 1926, and Hedwigia, **70**: 85. 1930, and Bull. Trim. Soc. Myc. Fr. **49**: 324. [1934]; Donk, Nederl. Kruidk. Archief. **1930**: 80. 1930; Lindau & Ulbrich, Die höhern pilze, p. 118. 1928; Jørstad, Kgl. Norske Vid. Selsk. Skrif. **1936** (10): 13. 1937; Skovsted, Compt. rend. trav. Lab. Carlsberg Ser. Phys. **25**: 407. 1956; Eriksson, Symb. Bot. Upsal. **16** (1): 119. 1958; and others); and in temperate North America (Burt, Missouri Bot. Gard. Ann. **7**: 123. 1920; Overholts, Bull. Torr. Bot. Club. **66**: 531. 1939; Nobles, Can. J. Botany, **34**: 119. 1956; and others), and in Eastern U.S.A. under *P. duplex* (Burt, Missouri Bot. Gard. Ann. **12**: 298. [1926]) and under *Corticium overholtsii* (Burt, Missouri Bot. Gard. Ann. **13**: 245. 1926; Overholts, Mycologia **21**: 281. 1929).

Peniophora pini (Schleich. ex Fr.) Boidin subsp. ***pini*** (A in Figs. 6–24)

Thelephora pini Schleich. ex Fr. Syst. Myc. **1**: 443. 1821.
Stereum pini (Schleich. ex Fr.) Fr. Epicr. p. 553. 1838.
Xerocarpus pini (Schleich. ex Fr.) Karst. Bidr. Kann. Finl. Natur. Folk, **37**: 135. 1882.
Sterellum pini (Schleich. ex Fr.) Karst. ibid. **48**: 405. 1889.
Peniophora pini (Schleich. ex Fr.) Boid. Rev. Myc. **21**: 123. 1956.

SPOROCARPS (A in Figs. 6–20)

FRUIT BODIES (Figs. 6-A, 7-A) discretely orbicular to confluent, to somewhat effused; smooth or irregularly tuberculate to cerebrally convolute, frequently raised or reflexed in age. COLOR variable, "first reddish, then blueish grey or almost blue-black when fresh, when dry pure grey or with a tint of violet, when old becoming brown" (Eriksson 1958). "Sterile MARGIN first distinct, white or pale rose, at last disappearing (when the radial growth has ceased)" (Eriksson 1958). SURFACE more or less pruinose. TEXTURE ceraceous to indurated and crumbly.

In SECTION, as described for the aggr. CONTEXT (A in Figs. 8–10) composed of a pronounced subiculum, more or less horizontal, of intertwined, rarely clearly parallel hyphae changing almost indistinguishably into the transition area leading to the erect subhymenium and hymenium; depth (less hymenium) ca. 250 to (?) 400 μ.

HYPHAE (Figs. 14-A, 19-A) separable, brittle, not embedded in a matrix; 4–11 μ in diameter, with walls of all hyphae usually thickened, typically swollen, frequently to as much as 4.5 μ in width; outline firm, or unevenly eroded, or cross-striate. Most hyphae with hyaline walls, except in dark zones where entire thickness of wall may be browned, or only outer layer. Swollen, attenuate, contorted, or dichotomously branched tips of brown-walled hyphae (Figs. 15-A, 18-A) sometimes frequent in subhymenium or hymenium.

GLOEOCYSTIDIA (A in Figs. 11, 12, 20) generally abundant, most regularly present in upper subicular, transition, and subhymenial areas; variable in shape (as described for aggr.) but typically strongly vesiculose to globoid (Figs. 11-A; 20-A-7, -8) with or without a narrowed stalk and hyphal rostrum; full length as much as 70 μ, expanded portion varying in length from 17 to 55 μ, in width from 7 to 27 μ.

CYSTIDIA (Figs. 13-A, 17-A) abundant to rare; wall of stalk rarely thickened to any extent; full length as much as 35 μ; apex a crystal-encrusted cap, 7–12 × 5–7 μ.

BASIDIA flexuous-clavate, ca. 5–6 μ broad at the apex. BASIDIOSPORES (Fig. 16-A) cylindrical, straight to almost reniform, 6.5–8.8 × 2–3 μ.

Hosts

Pinus spp., mainly, though other conifers reported: on fallen or dead still-attached branches, or trunk.

Distribution

Cf. above, the Eurasian references under *P. pini* aggr.: Britain, through continental Europe and into Siberia, as far east as Minusinsk and Mongolia.

Specimens Examined

Czechoslovakia: on *Pinus uncinatus*, Soběslav-Blata, April 4, 1953, F. Kotlaba (Czech. Union Dept. Agric. *40949*)—TRTC.
France: on *Pinus sylvestris*, Rhône, June 15, 1959, J. Boidin (LY *3181*)—DAOM *53382*.
Sweden: on unnamed pine: Danmark Parish, May 4, 1933, S. Lundell (ex Fl. Suec.)—TRTC; on *Pinus sylvestris*: O. Karup Parish, June 17, 1947, J. Eriksson (*1749*—ex Fung. Suec.)—DAOM *31310* and TRTC; Stockholm, March 12, 1905, L. Romell (*1121*—ex S)—TRTC; Värnamo, Sept. 11, 1958, B. Eriksson—DAOM *53381*.

Cultures

Key pattern: 2 2 1 1 1 2 2 2 2 2 2 (A in Figs. 21–24)

Growth Characters

Rate of growth moderately rapid, plates covered in 3 weeks (8.3–8.7 cm at 2 weeks). Advancing zone even, appressed. Mat (Fig. 21-A) appressed, white, nearly translucent, at first densely flecked around the inoculum with slightly raised, small, loosely arranged cottony tufts in newest part, later the flecks developing into small tufts, slightly raised, compactly cottony, becoming more numerous as the culture grows until they begin to coalesce in the oldest part; cottony-felty mycelium growing on side of plate as a ring-like mass protruding slightly inwards and most abundant above inoculum, tinged with pale brown (7.5 YR 6/6) in several places. Reverse unchanged. Faint fragrant odor suggesting spearmint.

Hyphal Characters

Advancing zone: hyphae (Fig. 23-A-1) hyaline, nodose-septate, 2.4–5.6 μ in diameter, with groups of short branches frequently found at the ends of hyphae. Aerial mycelium: (*a*) hyphae as in the advancing zone, soon developing narrow, frequently branched hyphal ends (Fig. 23-A-2) about 1.0 μ diameter, tapering to a fine point; (*b*) as the culture matures, thin-walled hyphae developing thickened walls (Fig. 23-A-3), these eventually gelatinizing into a thick outer sheath leaving only a narrow staining lumen, rarely roughened or frayed as though the outer surface of the wall had broken down; hyphae becoming cemented into strands to a certain degree (though less decidedly than in subsp. *duplex*, cf. below) forming an indistinct branching and anastomosing network; (*c*) numerous minute coiled tips (Fig. 23-A-4) present, the details indistinct; (*d*) occasional single strands of hyphae (Fig. 23-A-5) distinct because of their diameter (up to 7.2 μ), which is somewhat greater than that of the other hyphae, as well as their firmly thickened walls and infrequent branches and septation; (*e*) gloeocystidia (Fig. 24-A) numerous to infrequent, varied in shape, some plates producing many which are elongate (Fig. 24-A-1, -2), apparently formed when a hyphal tip or segment becomes oil-filled, rarely as narrow (1.6–3 μ) as in *P. pseudo-pini*, usually enlarged to a diameter of 3.2–6.4 μ, and up to 160 μ in length, or irregular in shape, or spathulate, broadly elongate (Fig. 24-A-3, -4), 19.2–48.0 μ in length, 6.4–11.2 μ in diameter; positive or negative in sulphuric-benzaldehyde.

Cultures Examined (from fruit bodies)

France: on *Pinus sylvestris*, Rhône (LY *3181*)—DAOM *53382*.
Sweden: on *P. sylvestris*, Värnamo—DAOM *53381*.

Peniophora pini subsp. ***duplex*** (Burt) stat. nov. (B in Figs. 6–24)

Peniophora duplex Burt, Missouri Bot. Gard. Ann. **12**: 298. [1926]. (!!)
Corticium overholtsii Burt, ibid. **13**: 245. 1926. (!!) (synonymy established by Rogers and Jackson, Farlowia **1**: 296. 1943).

Sporocarps (B in Figs. 6–20)

Fruit bodies (Fig. 6-B) discretely orbicular to broadly effused; typically smooth to occasionally umbonate or irregularly tuberculate; usually appressed; rarely slightly reflexed. Color variable but generally paler than other members of the aggr., white to cream to clay to flesh or grey, to greyish lavender or rosy brown; usually almost concolorous, only gradually fading outward, rarely with an abruptly lighter margin. Margin (Fig. 7-B) of actively growing fructification typically white fimbriate, sometimes on radial fingering; in age, vernicose and more or less entire; usually appressed at least basally, sometimes shelled away from substrate. Surface more or less pruinose, usually smooth, sometimes spongy just behind edge, occasionally so throughout. Texture soft-ceraceous to papery-crustose.

In SECTION entirely colorless or browned in patches or zones. CONTEXT (B in Figs. 8–10) composed of a pronounced horizontal subiculum of clearly parallel hyphae seen most distinctly in true radial section, a more or less abrupt transition area of oblique hyphae turning into an erect subhymenium and hymenium; depth (less hymenium) relatively shallow, only rarely exceeding 150 μ.

HYPHAE (Figs. 14-B, 19-B) 3–5.5 μ in diameter (though occasionally up to 7.2 μ broad in subiculum) with walls thin or only slightly thickened, or swollen to as much as 2.5 μ; occasionally some basal hyphae of subiculum with eroded or cross-striate walls; matrix in which subicular hyphae seem to be embedded more or less obvious. Most hyphae with hyaline walls except in dark-colored patches (bracketed in Fig. 15-B) where browning, typically due primarily to resinous material being deposited between the hyphae or penetrating the protoplast, may also color walls and hyphal tips individually, but only in close association with a generalized zone or patch of brown.

GLOEOCYSTIDIA (B in Figs. 11, 12, 20) rare to abundant, predominantly in the transition, subhymenial, and hymenial areas; variable in shape (as described for the aggr.) but most typically elongate (Fig. 12-B; 20-B-1 to -6), flexuous, cylindrical, pyriform, or obpyriform, subconical or subfusoid; variable in size of expanded portion: in length 12.5–70 (–100) μ, in width 5.5–20 μ, but when vesiculose in the ratio of less than 2 to 1, only occasionally over 12 μ in diameter.

CYSTIDIA (Figs. 13-B, 17-B) rare to abundant; wall of stalk thin, emphasized or thickened, but very rarely to the point of capillarity of the lumen; full length as much as 50 μ or more, though frequently shorter; crystal-encrusted apex 7.5–30 × 4–10.5 μ.

BASIDIA (Fig. 16-B) flexuous clavate, ca. 4.5–7 μ broad at the apex. BASIDIOSPORES (Fig. 16-B) cylindrical. straight to almost reniform, (5–) 6–7.8 (–9) × (1.7–) 2.2–2.8 (–3.3) μ.

HOSTS

Pinus spp

DISTRIBUTION

North America: from Ontario, south along the east coast of the United States, as far south as Florida State.

SPECIMENS EXAMINED

Ontario: on *Pinus banksiana*, Petawawa: Sept. 19, 1955: S. Gibson—DAOM *31784*; *31785*; *31787*; *31788*; *31789*; *31790*; M. K. Nobles—DAOM *31786*; *31791*; on *P. resinosa*, Petawawa, Sept. 19, 1955, M.K.N.—DAOM *31792*; on *P. sylvestris*, Simcoe Co., June 6, 1953, R. F. Cain—DAOM *30466-B*.

Connecticut: on *P. rigida*, Windsor, Sept. 2, 1935, H. G. Eeno (*F.P. 81246*)—TRTC.

Florida: on *Pinus* sp., Gainesville, Nov. 1, 1932, W. A. Murrill (*F. 18605*)—TRTC.

Georgia: on *P. taeda*, Athens, Nov. 18, 1938, G. E. Thompson (*1264*)—TRTC.

Maryland: on *P. virginiana*, Anne Arundel Co., Aug. 15, 1937, J. A. Stevenson (BPI *71189*)—TRTC.

Massachusetts: on *P. sylvestris*, Hamilton, Oct. 4, 1940, D. H. Linder and D. P. Rogers (D.P.R. *3177*)—TRTC; on *P. rigida*: Wakeby, March 16, 1927, R. C. Hall (Overh. *10608*; BPI *16995*)—PAC; Pembroke, June 6, 1937, D.H.L.—TRTC; on *P. ponderosa*: Hamilton: Sept. 7, 1928, J. R. Hansbrough (*F.P. 84038*)—DAOM *31311* and TRTC; July 26, 1928, P. Spaulding (*F.P. 81133*)—TRTC; Petersham, Aug. 10, 1928, J.R.H. (*F.P. 82464*)—DAOM *31312* and TRTC; on *P. strobus*, Hamilton, July 18, 1937-a-1, H. S. Jackson—TRTC.

New Jersey: on *Pinus* sp., Whitesville, Sept. 12, 1905, C. L. Shear (BPI *71146*)—TRTC.

New York: on *P. austriaca* (cult.), Shelter Island, Oct. 10, 1910, W. G. Farlow—FH—**type**; on *P. rigida*, Glens Fall, Sept. 9, 1932, J.R.H. (*F.P. 81143*)—TRTC; on unnamed conifer: Pack Forest: Sept. 5, 1958, A. R. Slysh (*597*)—DAOM *53383*; (*611*)—DAOM *53384*.

Pennsylvania: on *P. rigida*: Center Co., Oct. 5, 1918, L. O. Overholts (*4656*; MO *57155*)—BPI and PAC (**type** of *C. overholtsii*); State College, May 14, 1933, W. L. White (*1197*) and P. D. Keener—TRTC; Huntington Co.: Feb. 24, 1934, A. P. Kelley and W.L.W. (*1277*)—TRTC; May 5, 1935, W.L.W. (*1654*)—TRTC; Aug. 15, 1934, L.O.O. (*17601*)—TRTC; Clinton Co.: May 13, 1931, I. Mounce and L.O.O. (*13901*)—TRTC; May 3, 1931, L.O.O. and I.M.—DAOM *F1699* and TRTC; on *P. strobus*: Huntington Co.: Oct.18 (19?), 1931, L.O.O. (*13788*)—DAOM *F3802* and TRTC; Sept. 18, 1932, W.L.W. (Overh. *14800-A*)—DAOM *F2588-A* and TRTC; on *P. sylvestris*, Greenwood Furnace, Nov. 26, 1927, P.S. and L.O.O. (*10754*)—DAOM *31313* and TRTC; on *P. virginiana*, Huntington Co., Dec. 2, 1928, L.O.O. (*11363*)—TRTC and PAC.

CULTURES

KEY PATTERN: 2 2 1 1 1 2 2 2 (1,2) 2 (1,2) (B in Figs. 21–24; Fig. 22)

GROWTH CHARACTERS

RATE OF GROWTH rapid to moderately rapid, plates covered in 2 to 3 weeks. ADVANCING ZONE even, slightly raised or, rarely, appressed. MAT variable: forming either a thin, appressed, floccose-woolly mycelium that is patchy or indistinctly zonate, tinted reddish brown (7.5 YR 4/8) from the color within the agar, the mycelium itself tinged brown (7.5 YR 6/6) in spots—OR growing into an appressed floccose-reticulate mycelium, i.e. mat forming a mesh-like pattern on the surface of the agar, or a thin parchment-like film; either type of mycelium usually covering entire surface of plate, or covering only older part while the newer mycelium remains woolly-felty. Most mats (Fig. 20-B) producing raised balls or tufts concentrated near the inoculum or scattered over the surface of the mat, furry or cottony or tufted, often tinged with pale or medium brown (7.5 YR 6/6; 10.0 YR 7/6–5/4); most mats with woolly to felty mycelium thin and scattered or in thick patches or tufts growing on the sides of the plates, often tinged the same brown color. REVERSE unchanged, or with scattered minute brown dots (7.5 YR 5/6), or tinted reddish brown (5.0 YR 6/8), or a patchy dark brown (2.5 YR 3/6) and reddish brown (10.0 R 4/6). ODOR lacking.

HYPHAL CHARACTERS

ADVANCING ZONE: hyphae (Fig. 23-B-1) hyaline, thin-walled, nodose-septate, 2.4–4.8 μ diameter, soon becoming frequently branched, the branches short and stubby and often tapered at the end. AERIAL MYCELIUM: (*a*) hyphae as in the advancing zone; (*b*) myriads of very narrow branches (Fig. 23-B-2), 0.8–1.6 μ diameter, developing near the edge of the mat in most cases, tapered to a fine point, often disappearing as culture matures; (*c*) hyaline hyphae gradually losing contents, becoming non-staining and arranged in strands that usually form a distinct branching and anastomosing mesh-like NETWORK (Figs. 22; 23-B-3); (*d*) relatively broad straight hyphae (Fig. 23-B-6), up to 8.0 μ diameter, scattered throughout the mat, developing only slightly thickened walls or walls so strongly thickened that only a capillary lumen remains, the inner thickening often very uneven or wavy; (*e*) tips of branches losing contents and often forming a small tight coil (Fig. 23-B-5), the details not distinct; (*f*) mature hyphae acquiring slightly thickened refractive walls and often becoming gelatinously modified (Fig. 23-B-4), with an inner solid layer surrounded by a gelatinous sheath that causes their outline to be indistinct and that apparently cements them together into strands, rarely with the outer sheath roughened and frayed in appearance; (*g*) GLOEOCYSTIDIA (Fig. 24-B) lacking to infrequent, irregular in shape, or narrow and elongate, or, rarely, subglobose or broadly elongate; the latter type found exclusively in the tufts where the culture appears to be preparing to fruit, the other types also most frequently found in the tufts; 11.2–72.0 μ in length, (3.2–) 5.6–12.8 μ in diameter; negative in sulphuric-benzaldehyde except in some of the tufts where the culture may be attempting to fruit; (*h*) KNOTS (Fig. 23-B-7), when present, most numerous in raised tufts, formed by the tight coiling of one hyphal strand around another; (*i*) hyphal segments observable, scattered through the mycelium, with dense, homogeneous content, yellow-oily in appearance.

CULTURES EXAMINED (from fruit bodies)

Ontario: Petawawa: on *Pinus banksiana*—DAOM *31784* to *31791* inclusive; on *P. resinosa*, DAOM *31792*.

New York: Pack Forest: on unnamed conifer—DAOM *53383*; *53384*.

Peniophora pseudo-pini Weres. & Gibson nom. nov. (C in Figs. 6–24)

Phlebia cervina Overholts, Mycologia, **22**: 240. 1930. (!!) (non *Peniophora cervina* (Thüm.) Höhn. & Litsch. K. Akad. Wiss. Wien Math.-Nat. Kl. Sitzungsb. **115** (1): 1584. 1906, quod est nomen nudum?)

= *Stereum pini* sensu Auctt. Americanorum

SPOROCARPS (C in Figs. 6–20)

FRUIT BODIES (Fig. 6-C) discretely orbicular to confluent to somewhat effused but not broadly so; typically distinctly umbonate at one or several points, to irregularly tuberculate to cerebrally convolute, frequently raised or reflexed in age. COLOR variable, cream or rose-tan or flesh, to rosy brown to brown, to dusky or brownish purple, generally darkest in color centrally, particularly at umbos, usually abruptly lighter toward edge; rarely concolorous throughout. MARGIN of actively growing fructification (Fig. 7-C) typically white fimbriate, often with pronounced radial fingering outward; in age, still fingered but lacking fimbriation, or subvernicose, or abruptly entire, raised or down- and in-curved. SURFACE more or less pruinose. TEXTURE soft-ceraceous to subindurated or tough.

In SECTION, entirely colorless or browned as described for the aggregate. CONTEXT (C in Figs. 8–10) depth (less hymenium) typically 175–350 μ; SUBICULUM typically looking as if hyphae embedded in a matrix, composed of clearly parallel and mainly horizontal hyphae (in true radial section); TRANSITION AREA more or less gradual.

HYPHAE (Figs. 14-C, 19-C) 3.5–7 μ in diameter (occasionally up to 9.5 μ broad), with walls only slightly thickened or swollen to as much as 3 μ in width; subhymenial hyphae (Fig. 19-C-3, -4) usually with walls considerably narrower than those of the subiculum (Fig. 19-C-5 to -9) where hyphal outlines may become indefinite through gelatinization, erosion, or cross-striation. Most hyphae with hyaline walls except in darker-colored patches; but individual brown-walled hyphae traversing a predominantly hyaline area, with swollen, attenuate, or contorted or dichotomously branched tips in subhymenium or hymenium (Figs. 15-C, 18-C), when present, are strongly diagnostic of this taxon.

GLOEOCYSTIDIA (C in Figs. 11, 12, 20) generally abundant, but varying from rare to very numerous; observed throughout the context and in the hymenium, but typically and most regularly in the upper subicular, transition, and lower subhymenial areas; variable in shape (as described for *P. pini* aggr.), but most typically strongly vesiculose to globoid (Figs. 11-C; 20-C-3, -5, -6), on narrowed stalk, with or without a hyphal rostrum; variable, also, in size of expanded portion: in length up to 50 μ, though averaging ca. 25 μ, in width as much as 20 μ or over, but generally 10–15 μ.

CYSTIDIA (Figs. 13-C, 17-C) abundant to rare, projecting or not; when mature, wall of stalk thin, emphasized, or thickened, swollen sometimes to the point of capillarity of the lumen; where brown hyphae frequent, sometimes arising from these with brown stalks; full length as much as 60 μ though frequently shorter, particularly in the hymenium; apex sometimes capped only with a scatter of crystals but characteristically heavily encrusted, from 8 × 5.5 μ to as much as 40 × 15 μ.

BASIDIA flexuous clavate, ca. 4.5–5.5 μ broad at the apex. BASIDIOSPORES (Fig. 16-C) cylindrical, straight to almost reniform, (5.5–) 6–7.2 (–7.7) × (1.7–) 2.2 (–2.5) μ.

HOSTS

Pinus spp. (rarely others conifers); usually attached dead branches or dead saplings.

DISTRIBUTION

Temperate North America.

SPECIMENS EXAMINED

Alberta: on *Pinus contorta* var. *latifolia*: Strachan, July 17, 1953, V. J. Nordin (*A-53-31*)—DAOM *30920*; Water Valley, Aug. 27, 1953, M. K. Nobles and V.J.N. (*F.P. 922*)—DAOM *30521*.

British Columbia: on *P. contorta*: Quesnel: July 22, 1949, W. G. Ziller (DAVFP *5275*)—DAOM *22719*; (DAVFP *5225*)—DAOM *30872*; Summit L., Prince George, Sept. 1, 1950, W.G.Z. (DAVFP *6636*)—DAOM *30873*.

Ontario: on coniferous twigs: Kindiogami L., July 25, 1950, D. A. Quirke (*420*)—DAOM *52335*; on unidentified pine; Constance Bay, June 10, 1933, J. W. Groves *et al.*—DAOM *F2133*; on *Pinus strobus*: Timagami: Sept. 1, 1935, L. O. Overholts—TRTC *8278*; Aug. 2, 1937, H. S. Jackson—TRTC *12156*; July 28, 1938, R. F. Cain—TRTC *13819*; E. Maple, Nov. 2, 1935, H.S.J.—TRTC *8279*; N. Richmond Hill, May 16, 1937, H.S.J.—TRTC *11122*; Algonquin Park: Sept. 13, 1939, R.F.C.—TRTC *14417*; Aug. 24, 1939, R.F.C.—TRTC *14773*; Simcoe Co., June 4, 1951, R.F.C.—TRTC *23602* and DAOM *30239*; Blakeney, July 29, 1952, M.K.N.—DAOM *30124*; Petawawa, Aug. 19, 1951, B. W. Dance (*24*)—DAOM *31181*; on *P. sylvestris*: Simcoe Co.: June 6, 1953, R.F.C.—DAOM *30466-A*; TRTC *24352*; on *P. banksiana*: North Bay: Sept. 21, 1955: S. Gibson—DAOM *31793*; *31794*; *31795*; *31796*; Petawawa, Sept. 9, 1939, H.S.J.—TRTC *14312*; on *P. resinosa*, Simcoe Co., Oct. 16, 1951, R.F.C.—TRTC *23561* and DAOM *22982*; on *Abies balsamea*, Pt. Alexander, Sept. 13, 1939, H.S.J.—TRTC *18059*.

Quebec: on *Pinus strobus*, Gatineau, July 26, 1931, H. T. Gussow—DAOM *F2133*.

Massachusetts: on *Pinus ponderosa*, Ipswich, Jan. 31, 1929, W. H. Snell *et al.* (*763*) (Overh. *11484*)—PAC—**type**; on *P. strobus*: Milton, Nov. 30, 1935, D. H. Linder *et al.* (W. L. White *2191*) (CUP *25045*)—TRTC; Hamilton: July 18, 1937, G. D. Darker (*6347*)—TRTC; July 18, 1937-a-2, H.S.J.—TRTC; July 18, 1937-*b*, H.S.J.—TRTC; on unidentified *Pinus* sp., Hamilton, July 18, 1937, H.S.J. and D.H.L.—TRTC.

New York: on *Pinus resinosa*, Canadice L., Aug. 4, 1938, J. R. Hansbrough (*F.P. 84856*) (as *Peniophora duplex*)—TRTC.

Pennsylvania: on *Pinus strobus*: State College, Aug. 27, 1933, W.L.W. (*1238*) and P. D. Keener—TRTC; Huntington Co., Sept. 18, 1932, W.L.W. (Overh. *14800-B*)—TRTC, DAOM *F5226* & *F2588-B*; Feb. 19, 1933, L.O.O. (*15493*) and R. W. Davidson—DAOM *F6572* and TRTC; June 12, 1938, L.O.O. (*21143*)—TRTC; July 10, 1932, W.L.W. (Overh. *14459*)—TRTC.

CULTURES

KEY PATTERN: 2 2 1 1 1 2 2 2 (1,2) 2 1 (C in Figs. 21–24)

Growth Characters

Rate of growth moderately rapid, plates covered in 3 weeks, or occasionally in 2 weeks. Advancing zone even, slightly raised or, rarely, appressed. Mat white at first, frequently at 2 weeks developing minute droplets of brown resinous material (7.5 YR 4/4) on the surface in small irregular areas or along short lines or on the surface of the mycelium growing against the side of the petri plate; after 3 or 4 weeks, mycelium itself becoming buff to medium brown (10.0 YR 7/6, 10.0 YR 5/4, 7.5 YR 5–6/6) in spots or patches, the color spreading somewhat as the culture matures, or at 6 weeks minute brown dots (7.5 YR 5/4) possibly spattered over surface. Mat (Fig. 21-C) variable; slightly raised at first, then appressed, or collapsed or appressed only in older mycelium; later cottony-floccose or woolly-floccose or abundantly loose cottony, often showing a somewhat reticulate arrangement of the hyphae producing a spongy appearance when the culture is young; thin and translucent or thick enough to be opaque, rarely becoming densely farinaceous and sometimes developing patches where the aerial mycelium seems to be disappearing and leaving the agar bare, with suggestion of radiate striation occasionally noticeable; mycelium at first abundant, cottony or woolly, later felty and tinged with brown (7.5 YR 5/6), growing on sides of petri plates. Reverse brown (2.5 YR 4/6) around edges or in patches or, frequently, speckled with minute brown dots (5.0 YR 3/4; 2.5 YR 3/6). Odor of coconut distinct in some cultures, lacking in others.

Hyphal Characters

Advancing zone: hyphae (Fig. 23-C-1) hyaline, nodose-septate, 3.2–4.8 μ diameter. Aerial mycelium: (*a*) hyphae as in advancing zone, soon forming short branches (Fig. 23-C-2), stubby in appearance or with slightly tapered tips; (*b*) certain strands of hyphae standing out, relatively broad (Fig. 23-C-3), straight, infrequently septate or branched, eventually losing their cell contents and becoming slightly thick-walled, up to 6.4 μ diameter; (*c*) thin-walled hyphae (Fig. 23-C-4) in some cultures 'bumpy' or irregular in outline; (*d*) brown-colored areas of mat containing many hyphae with yellowish brown contents, occasional segments becoming opaquely homogeneous, yellow oily; (*e*) in maturing, thin-walled hyphae acquiring slightly thickened walls (Fig. 23-C-5), often surrounded by a gelatinous sheath obscuring the outline, rarely becoming roughened on the outer surface; (*f*) hyphal gloeocystidia up to 120 μ in length, very narrow (Fig. 24-C-1), 1.6–3.2 μ diam., or broader (Fig. 24-C-2, -4), often forked or with short branches, occasionally with central or lateral swelling or with empty, non-staining tips as though cytoplasm had receded; or gloeocystidia irregular in shape to globose (Fig. 24-C-3, -5), 5–20 μ in diam., all deeply staining, with oily granular contents, all negative in sulphuric-benzaldehyde; the hyphal gloeocystidia always present, the swollen type rarely seen; (*g*) occasional groups of short branches (Fig. 23-C-7) standing out when a gelatinous 'halo' is laid down on the outer surface of the hyphae; (*h*) tips of non-staining short branches frequently becoming coiled (Fig. 23-C-6), outline indistinct; (*i*) occasional knots (Fig. 23-C-8) formed by a hypha twining closely around another strand, or becoming tightly contorted, or swelling with short protruding branches from a deeply staining core.

Cultures Examined (from rot or stain)

Alberta: on *Pinus contorta* var. *latifolia*, Strachan—DAOM *31217*; Water Valley—DAOM *31218*; *31219*; on *Picea glauca*, Slave L.—DAOM *31215*.
British Columbia: on *Pseudotsuga taxifolia*—DAOM *31216*.
Ontario: on *Pinus strobus*, Timagami—DAOM *21017*; Barry's Bay—DAOM *31214*; on *P. banksiana*, Thunder Bay D.—DAOM *31220*.

(from fruit bodies)

Ontario: on *Pinus strobus*, Blakeney—DAOM *30124*; on *P. resinosa*, Simcoe Co.,—DAOM *22982*; on *P. banksiana*, North Bay—DAOM *31793*.

Acknowledgments

Our debt to Dr. M. K. Nobles is obvious: the project was a continuation of one of her problems, and she has been our constant consultant and mentor. We are indebted also to those who sent us collections for our use in culturing and interfertility studies: Prof. J. Boidin, Dr. J. Eriksson, and Dr. A. R. Slysh; for their ready cooperation, to the curators of the herbaria from which we received specimens on loan: Farlow, Pennsylvania State University, the U.S. National Fungus Collections, and the University of Toronto; for assistance in trying to trace the original description of *Xerocarpus cinereus* var. *cervinus* Thüm. (*Peniophora cervina* (Thüm.) Höhn. & Litsch.) to Mrs. I. M. Lamb, of Farlow, and Dr. K. H. Rechinger, Naturhistorisches Museum, Vienna;

and to the many with whom we have discussed matters generally or particularly pertaining to this problem, especially Drs. Åskell and Doris Löve, Dr. Ruth Macrae, and Prof. D. P. Rogers. The photographs were taken and plates prepared for publication by the Bio-graphic Unit, Research Branch, Canada Department of Agriculture.

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NOTES

CAEOMA FAULLIANA, SYNONYM OF THE TREE RUST FUNGUS MELAMPSORA ALBERTENSIS[1]

W. G. ZILLER[2]

In 1936, *Caeoma faulliana* Hunter (3, p. 118) was described as a new species of rust on alpine fir (*Abies lasiocarpa* (Hook.) Nutt.), because its pycnia differed distinctly in position and morphology from the pycnia of all other caemoid rust fungi known to occur on the needles of fir (*Abies* spp.). During an investigation of the rusts of hemlock (*Tsuga* spp.) and fir, and through the courtesy of Dr. Lillian M. Hunter, the writer examined (6, p. 114) and photographed (Figs. 4 and 5) part of Dr. Hunter's permanent mounts which contained sections of needles with pycnia and aecia from the type collection of *Caeoma faulliana*. The type collection itself was missing and believed to be lost. Since no herbarium specimens seemed to be in existence, an intensive search for the rust was made in 1958 at its type locality, Banff, Alberta, and at the same time of the year the type was collected: the beginning of July. This search yielded many specimens of *Caeoma* on alpine fir, but none bore the characteristically small, subcuticular pycnia of *C. faulliana*. Recently, the type collection was located and made available to the writer.

The Host

The type collection of *C. faulliana* is relatively ample, very uniform, and well preserved; it consists entirely of young shoots with needles (Fig. 1) approximately 10% of which bear pycnia (Figs. 4 and 5) and caeomata (Fig. 4). The following observations were made:

> Twigs immature, containing no secondary xylem; buds acute (over 25 buds examined); needles acute to obtuse at the apex, twisted and distinctly petiolate at the base; leaf scars as a rule slanted with respect to the axis of the shoot; stomata entirely hypophyllous. Resin canals marginal; vascular bundle as a rule undivided and surrounded by a well-defined, thick-walled endodermis.

These observations were considered in the light of Feustel's (1, p. 218) remarks on Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Fulling's (2) anatomical data on alpine fir, and compared with descriptions in five well-known floras. The type material itself (Fig. 1) was compared with other specimens including cross sections of the needles of Douglas fir and of fresh and preserved foliage of alpine fir (Figs. 3, 6, and 7). All observations confirmed the writer's original sight identification of the host as Douglas fir.

[1]Contribution No. 641, Forest Biology Division, Research Branch, Canada Agriculture, Ottawa.
[2]Forest Biology Laboratory, Victoria, British Columbia.

The Parasite

The following measurements were obtained from mature to overmature fructifications of infected needles:[3]

Pycnia 30–87μ wide × 40–94μ long × 30–50μ high (Figs. 4 and 5; 21 pycnia measured); pycniospores 1.5–2 × 3–4.5μ; aecia 0.10–0.27 mm wide × 0.33–1.15 mm long (Fig. 4; 25 aecia measured); aeciospores 18–22 × 21–26μ; spore walls 1.5–3.0μ, the bilaterally thickened side walls 4.5–6μ thick (Fig. 2; 30 spores measured); spacing from center to center of warts 0.75–1.5μ.

Comparisons make it evident that *Caeoma faulliana* represents the pycnial and aecial states of *Melampsora albertensis* Arth. The excessive bilateral thickening of the walls of many aeciospores (Fig. 2; compare ref. 5, p. 182) is ascribed to the fact that the caeomata of *Caeoma faulliana* were overmature at the time they were collected, on July 8, 1925. Spore walls, as a rule, become thicker with the advancing physiological age of the spores.

Apparently, the type of *Caeoma faulliana* has not been readily available and the identity of its host, determined by the collector as alpine fir, has not been questioned. The results presented herein show that *C. faulliana* is a synonym of *Melampsora albertensis*, a fungus causing one of the needle rust diseases of Douglas fir. Isotypes of *C. faulliana* are deposited in DAOM,[4] DAVFP, PUR, TRTC, and BPI.

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[3]Compare with Hunter's (3) diagnosis and illustration.
[4]For abbreviations of names of herbaria Lanjouw and Stafleu (4) are followed.

Received May 20, 1960.
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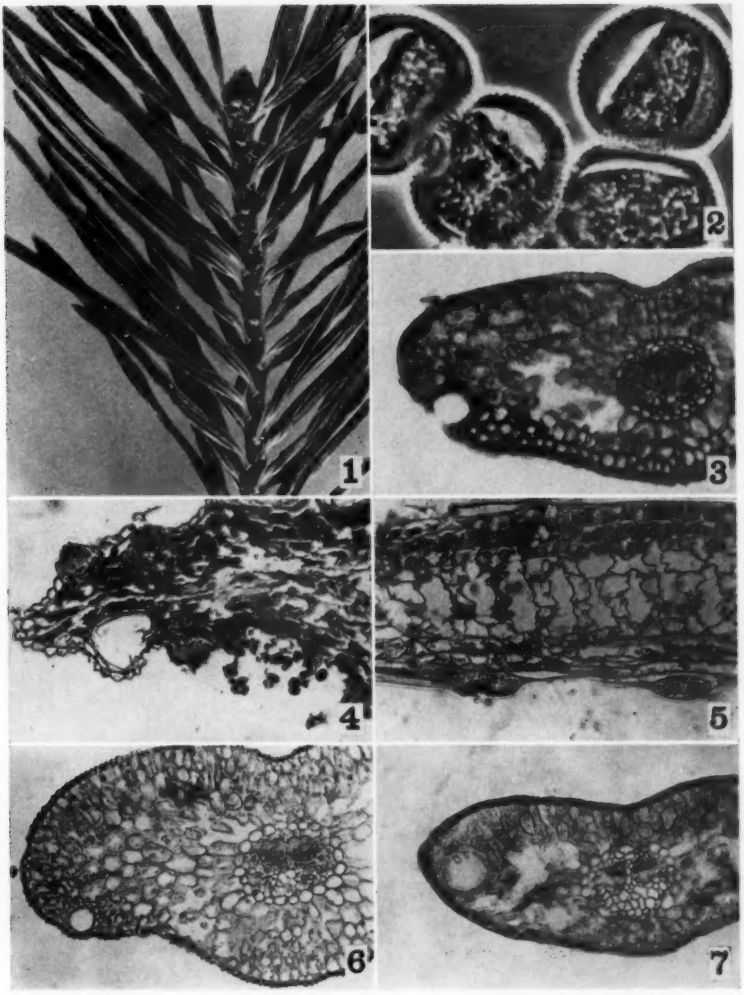
PLATE 1



FIGS. 1–5. *Caeoma faulliana*, from type collection. Fig. 1. Shoot bearing infected foliage; note acute bud, petiolate needles, and slanted needle scars; ×2. Fig. 2. Aeciospores; note bilaterally thickened spore walls; median optical section under phase contrast illumination; ×1000. Fig. 3. Cross section of healthy needle from a shoot bearing infected foliage; ×50. Figs. 4 and 5. Cross and longitudinal sections, respectively, of infected needles from Dr. Hunter's permanent mounts of *C. faulliana*; ×100. Figs. 6 and 7. Cross sections of healthy Douglas fir (Fig. 6) and alpine fir (Fig. 7) needles from regions of interior British Columbia; ×50.

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